

## ABSTRACTS

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## **Uptake of Nanoparticles of Cerium Oxide and Yttrium Oxide by *Acanthamoeba castellanii* (Protozoa) and *Daphnia magna* (Crustacea)**

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### **ABSTRACT**

Currently, nanoparticles are synthesized and used at an unprecedented rate for industrial, medical, and research applications. The use of cerium oxide nanoparticles (CeONP) and yttrium oxide nanoparticles (YtONP) results in their spread as contaminants into the environment. Once in the environment, CeONP and YtONP can be taken up by organisms in the food chain where they may pose a public health risk. In this study we determine whether *Acanthamoeba castellanii* and *Daphnia magna* uptake CeONP or YtONP from their environment and thereby play a role in the transmission of the nanoparticles. Using electron microscopy, organisms exposed to the nanoparticles were examined. Our results indicate that the nanoparticles are associated with cell and organelle membranes. These findings have implications for the health risks associated with environmental contamination by CeONP and YtONP.

### **INTRODUCTION**

In this study we determine whether protists and crustaceans play a role in the transfer of cerium oxide nanoparticles (CeONP) and yttrium oxide nanoparticles (YtONP) from the environment to other organisms within the aquatic food chain. *Acanthamoeba castellanii*, a common protist, and *Daphnia magna*, a planktonic crustacean, are important components in many aquatic ecosystems. Because acanthamoebae, such as *A. castellanii*, are aggressive feeders they consume inorganic and organic compounds from their environment, thereby serving as a link by transferring normally unavailable inorganic components to the food chain (Weekers et al. 1993). *D. magna* feeds on acanthamoebae and other protists found in lower trophic levels. Because of the high reproductive potential of *D. magna*, these planktonic crustaceans can substantially alter the structure and functioning of microbial food webs in freshwater ecosystems, such as acidic swamps, freshwater lakes, ponds, rivers and streams (Guisande 1993). *D. magna* and other aquatic crustaceans have the ability to filter particles of a variety of sizes including nanoparticles (Rosenkranz et al. 2009, Kim et al. 2010, Zhu et al. 2010). The authors hypothesized that CeONP or YtONP in

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the natural environment could be taken up by these organisms. Particles engineered at dimensions between 1-100 nm, are referred to as nanoparticles. Currently, nanoparticles are synthesized and used for industrial, medical, and research applications. CeONP are used as diesel fuel additives, in automotive catalytic converters, and are a by-product of many industrial processes, including the polishing of glass and semi-precious stones. CeONP also have potential uses for medical applications acting as an antioxidant (Elswaifi et al. 2009). Yttrium oxide and cerium oxide belong to the rare earth elements. Yttrium oxide nanoparticles (YtONP) are used in the manufacturing of cathode ray tubes for computer monitors and televisions and have potential medical applications due to their ability to act as antioxidants (Schubert et al. 2006, Cotton 2006, Okuyama et al. 2007, and Gilmore et al. 2008). The use of CeONP and YtONP results in their release into the environment (Biswas and Wu 2005, Chow et al. 2005) where they may exist in concentrations and forms that are toxic. This release may result from the process of their synthesis, as a by-product of their use, or from their indiscriminate disposal after use. Presently, CeONP and YtONP are released into the environment from diesel engine emissions, from improper disposal of automotive catalytic converters, and from improper disposal of old TV and computer monitors. These nanoparticles may then make their way into air, soil, or ground water (Biswas and Wu 2005, Chow et al. 2005). As with many of the engineered nanoparticles, CeONP and YtONP may also have toxic effects on humans and animals (Gatti and Montanari 2008). The toxicity of CeONP and YtONP has been recently investigated *in vivo* and *in vitro* (Gojova et al. 2007, Gatti and Montanari 2008, Andelman et al. 2009, Hardas et al. 2010). Toxic effects include reduced cell viability, increased cellular oxidative damage, and apoptosis. Effects also include vascular inflammation that may lead to pulmonary thromboembolism resulting in stroke or myocardial infarction. Chronic inflammation may also lead to rare earth pneumoconiosis or lung cancer (Gojova et al. 2007, Gatti and Montanari 2008, Andelman et al. 2009, Hardas et al. 2010, Lin et al. 2006). However, the routes of exposure of humans to these nanoparticles are poorly understood. Examples of routes of exposure of CeONP and YtONP may be through the contamination of organisms in the food-chain or through contamination of drinking water (Holbrook et al. 2008). In a typical food chain, there is usually a maximum of four or five trophic levels, although food chains in aquatic ecosystems frequently contain more levels than those in terrestrial ecosystems (Pimm and Lawton 1977). As organisms in lower trophic levels are consumed by those in a higher level, nanoparticles may become concentrated in top level consumers, level consumers, namely fish and animals that consume fish, including humans. Many protists, including acanthamoebae, are voracious feeders of organic and sometimes inorganic materials as they occupy the bottom of aquatic and some terrestrial ecosystems (Khan 2009).

*Acanthamoeba* spp. are free-living amoebae that are ubiquitous in aquatic and terrestrial ecosystems. Acanthamoebae exist as trophozoites, the feeding stage, and as cysts. Presently, 23 species of *Acanthamoeba* are reported and their biology and pathogenicity reviewed by Marciano-Cabral and Cabral (2003) and Khan (2009). At least three species of *Acanthamoeba* have been reported as parasites of animals, and humans (Marciano-Cabral and Cabral 2003 and Khan 2009). It is unknown whether CeONP or YtONP particles are taken up and concentrated by protists or by crustaceans in contaminated aquatic ecosystems. In this report we investigate whether CeONP and

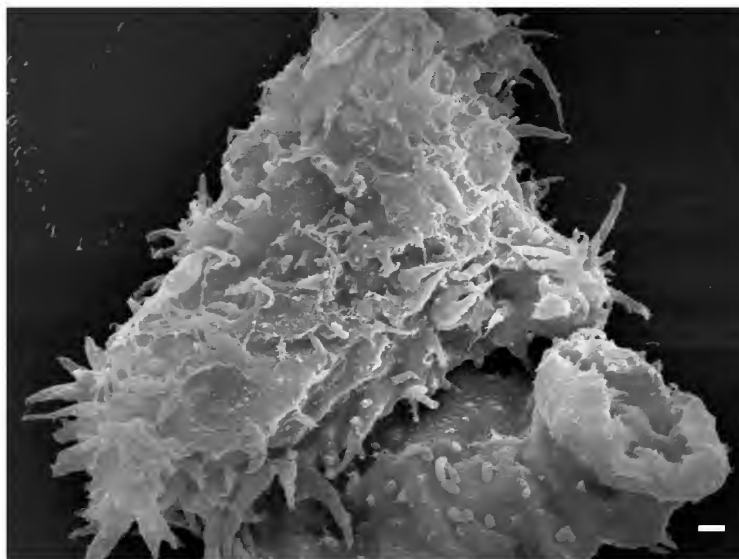


FIGURE 1. Scanning electron micrograph showing two trophozoites feeding stages of *Acanthamoeba castellanii*.

YtONP may be incorporated into the trophozoite *Acanthamoeba castellanii* (Fig. 1) and into the arthropod *Daphnia magna*, two integral components representing organisms at two different trophic levels of the aquatic food chain.

#### MATERIALS AND METHODS

**Exposure of Organisms to Nanoparticles:** CeONP and YtONP stock solutions were prepared by suspension of nanoparticles in distilled water. Samples of the nanoparticles suspended in distilled water were placed in a vortex apparatus for 5 minutes before and after preparation of the solution to minimize formation of nanoparticle aggregates. *D. magna* cultures were exposed to nanoparticles by the addition of the respective solution to make a final concentration of 10  $\mu$ M of CeONP or YtONP. *D. magna* viability was determined by observing motility and gill movement. Distilled water without nanoparticles was used in control samples. CeONP or YtONP were added to cultures of *A. castellanii* containing >95% trophozoites, making a final nanoparticle concentration of 100 nM. Equal amounts of distilled water were used for exposure of control samples. Cultures were incubated for 24 hours at 25°C, washed two times in Page's saline solution (Petry et al. 2006), then processed for electron microscopy using standard techniques. Control groups consisted of organisms treated with only distilled water. To define the appearance CeONP and YtONP alone, suspensions were used to prepare samples for observation by transmission and scanning electron microscopy.

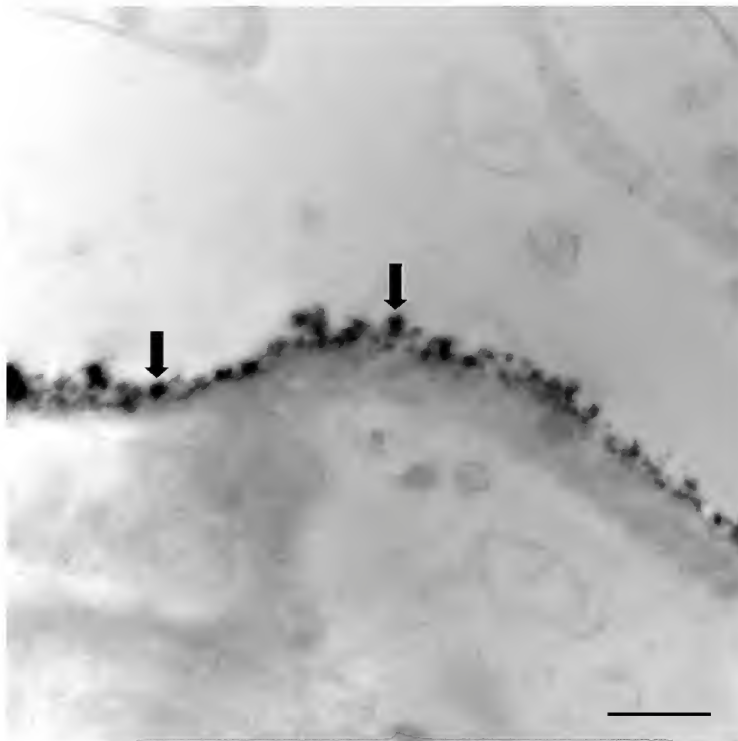


FIGURE 2. Outer membrane of *Acanthamoeba castellanii* demonstrating accumulations of yttrium oxide nanoparticles attached to outer surface. Arrows indicating yttrium oxide nanoparticles (Bar = 500nm ).

The appearance of CeONP and YtONP in cells was compared to those reported in other studies. Each experiment was performed twice including control groups. There were sixteen experimental group cultures; eight cultures of *A. castellanii* and eight cultures of *D. magna*. There were four control groups run for each species; two cultures each, exposed to CeONP for *D. magna* and *A. castellanii* and 2 cultures each, exposed to YtONP for *D. magna* and *A. castellanii*.

**Electron Microscopy:** After exposure of *A. castellanii* and *D. magna* to CeONP or YtONP, specimens were prepared for electron microscopic examination. *A. castellanii* was washed two times using Page's saline solution, centrifuged and fixed in 0.5% buffered glutaraldehyde at 4C for 72 hours. *D. magna* was pre-fixed in FAA fixative solution for 5 minutes at 4C and post-fixed in 0.5% buffered glutaraldehyde at 4C for

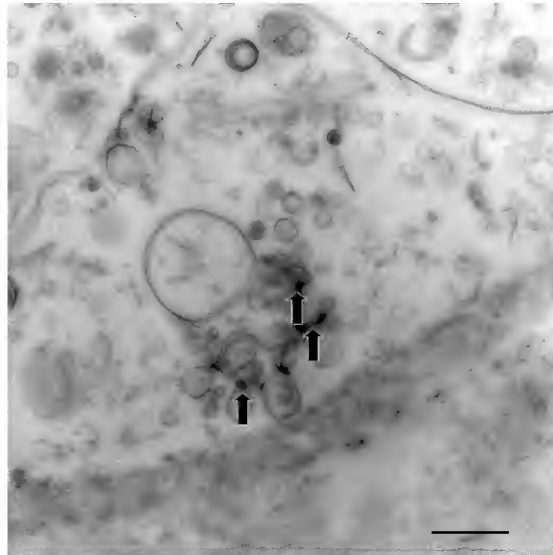


FIGURE 3. Golgi apparatus of *Acanthamoeba castellanii* demonstrating cerium oxide nanoparticles associated with Golgi vesicles. Arrows indicating cerium oxide nanoparticles (Bar = 500nm).

72 hours. *A. castellanii* and *D. magna* were then processed for ultra-thin sectioning and examination using transmission electron microscopy according to standard methods used by the Virginia-Maryland Regional College of Veterinary Medicine Morphology Services Laboratory. Electron micrographs of thin sections of *A. castellanii* and *D. magna* were examined for densely stained nanoparticles of CeONP and YtONP to determine if nanoparticle uptake had taken place. For scanning electron microscopy, samples of *A. castellanii* were isolated and processed according to standard methods used by the Virginia-Maryland Regional College of Veterinary Medicine Morphology Services Laboratory.

## RESULTS

Our results demonstrate that CeONP and YtONP can be readily taken up by *A. castellanii* and *D. magna*. We have also demonstrated that after uptake, CeONP and YtONP were associated with cell and organelle membranes in these organisms. *Acanthamoeba castellanii*: CeONP and YtONP were observed in association with cell and organelle membranes of *A. castellanii*. These nanoparticles were observed on the outer surface of the cell membrane (Fig. 2) and on the membranes of intracellular organelles, including Golgi apparatus (Fig. 3) and various vesicles (Fig. 4). Vesicles

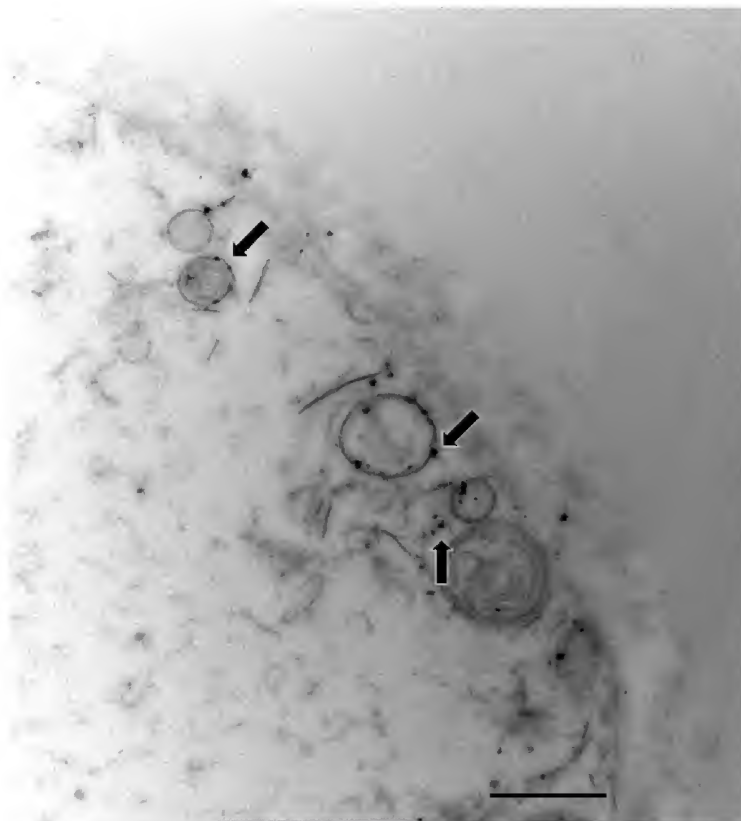


FIGURE 4. *Acanthamoeba castellanii* demonstrating yttrium oxide nanoparticles within organism outer membrane and organelle membranes. Arrows indicating yttrium oxide nanoparticles (Bar = 500nm).

containing nanoparticles were observed lining the inner surface of the cell membrane (Fig. 4). In addition, *A. castellanii* treated with YtONP revealed that the nanoparticles blanketed the outer cell surface and sometimes appeared as aggregates, some crystalline and some with ill-defined edges throughout the organism.

*Daphnia magna*: CeONP and YtONP were observed throughout the tissues of *D. magna*. These nanoparticles were observed in multiple organs including reproductive and digestive, and were also observed in gill filaments. Observation of both cross sections and longitudinal sections of gill filaments revealed a close association with



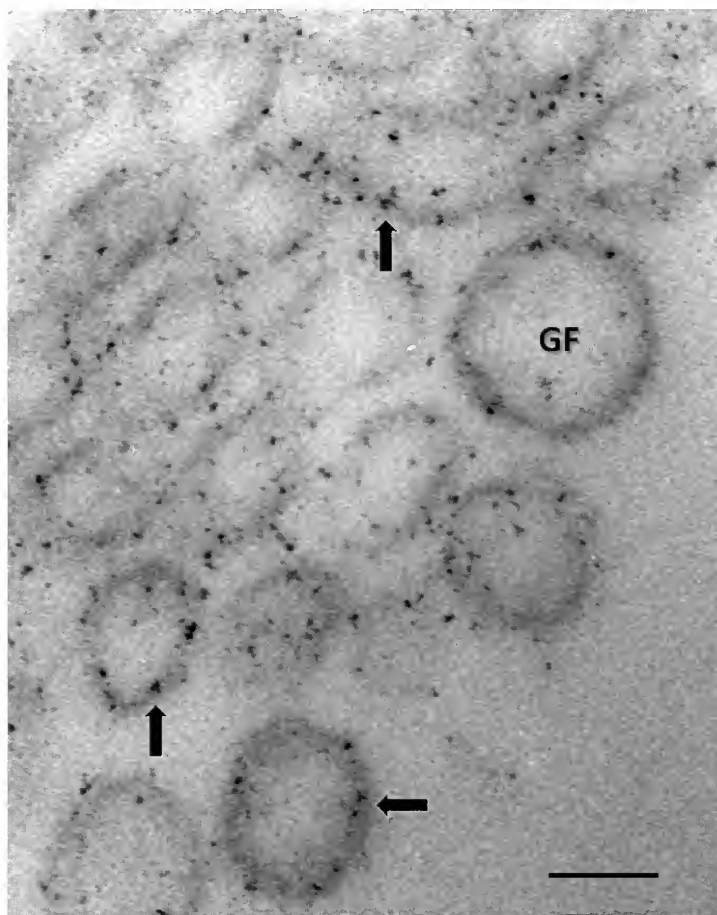


FIGURE 5. Cross sections of gill filaments (GF) of *Daphnia magna* showing yttrium oxide nanoparticles on gill filament borders. Arrows showing yttrium oxide nanoparticles on gill filament borders (Bar = 100nm).

YtONP (Fig. 5 and Fig. 6). YtONP were also observed in the space underlying the gill filaments (Fig. 6). CeONP were observed at the site of muscular attachment to the cuticle. At the sub-cellular level, CeONP and YtONP were observed in mitochondria, nuclei, muscle fibers, vesicles, and within the multilamellar bodies (Fig. 7). CeONP

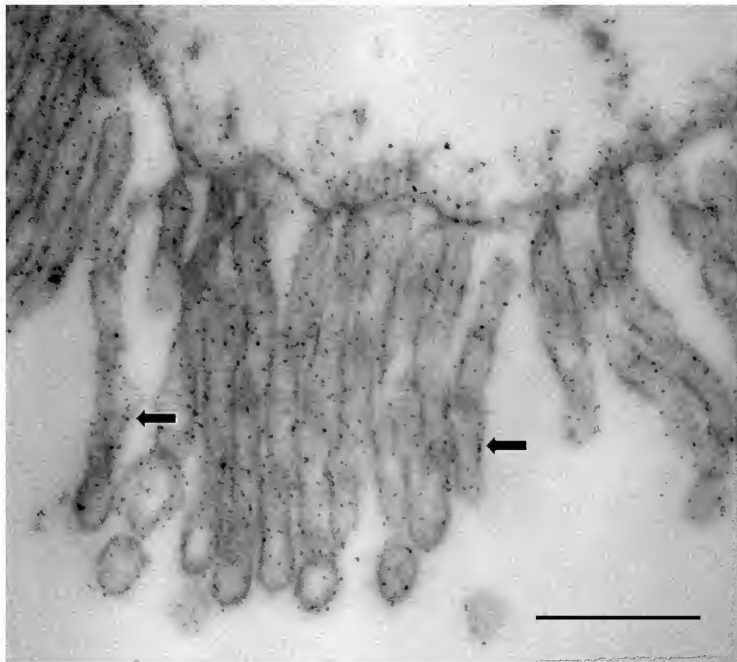


FIGURE 6. Longitudinal section of gill filaments of *Daphnia magna* demonstrating yttrium oxide nanoparticles along gill filament borders. Arrows showing yttrium oxide nanoparticles on gill filament boards (Bar 500 nm).

and YtONP were observed on the surface of various organelle membranes, on mitochondrial cisternal membranes, and on the membrane surfaces and internal contents of vesicles.

#### DISCUSSION

The observations of nanoparticles in both *A. castellanii* and *D. magna* indicate that nanoparticles are associated with cells and their membranes. The association of CeONP and YtONP with cellular membranes of *A. castellanii* and *D. magna*, may indicate the first step in the diffusion of the nanoparticles into the cell. The association of the nanoparticles with vesicles found along the cell surface (Fig. 4) may indicate that the nanoparticles are actively transported into the cells by endocytosis or possibly delivered through membrane channels to vesicles for further breakdown. Alternatively, the nanoparticles may diffuse passively through the membranes. The presence of proteins

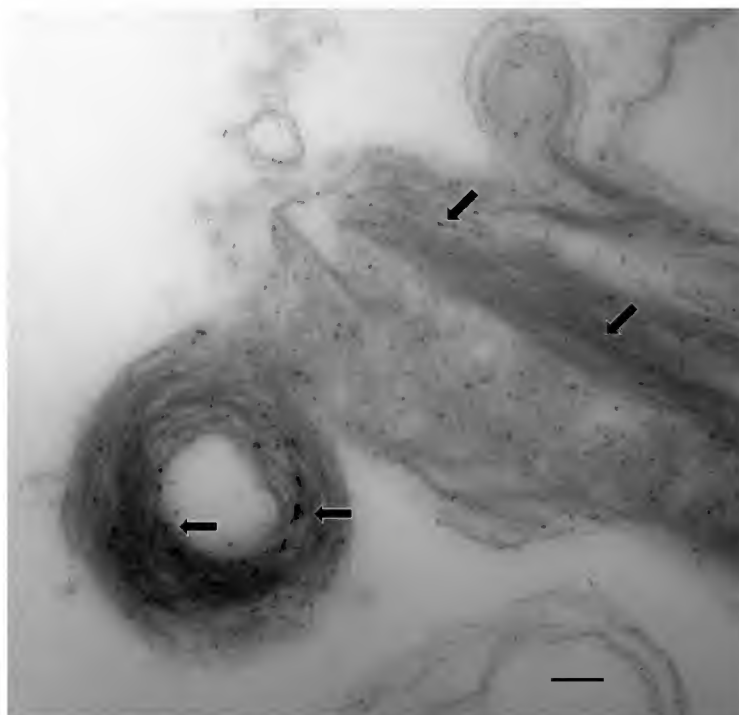


FIGURE 7. Multilamellar body and associated structures of *Daphnia magna* showing cerium oxide nanoparticles throughout. Arrows showing cerium oxide nanoparticles (Bar = 100nm).

on the cells surface of *A. castellanii* may play a role in enhancing the uptake of nanoparticles by coating the nanoparticles and enhancing phagocytosis (Andelman et al. 2009, Lynch et al. 2009). Both CeNOP and YtNOP were associated with cell and organelle membranes. However, the amount and distribution of YtNOP in cells appeared to be greater than that for CeONP.

Acanthamoebae and planktonic crustaceans are important components in aquatic food chains (Jürgens 1994, Zöllner et al. 2003). Acanthamoebae interact directly with bacterial and fungal decomposers, and with autotrophic and heterotrophic microplankton. Our observation of YtNOP in the gill filaments of *D. magna* (Fig. 5. and Fig. 6) supports the ability of these crustaceans to filter particles in the nanoscale range. The presence of *D. magna* may influence the food chain structure because they

consume microplankton from lower trophic levels. In turn they are consumed by fish, making *D. magna* a key factor in the propagation of trophic cascades from bacteria to fish (Jürgens 1994). It is possible in the natural environment that nanoparticles could be transferred to *Daphnia* following the ingestion of acanthamoebae containing nanoparticles; fish could then feed on these *Daphnia*. Fish containing nanoparticles may transfer them directly to humans who consume the fish, or indirectly through the feeding of forage-fish to livestock, which are then consumed by humans.

The ability of *A. castellanii* and *D. magna* to uptake CeONP and YtONP from their environment has implications on the organisms' ability to deliver nanoparticles to higher trophic levels, including human who consume fish species. The potential for transfer of CeONP and YtONP to humans through the food chain poses a significant public health risk (Biswas and Wu 2005, Chow et al. 2005, Gojova et al. 2007, Gatti and Montanari 2008, Gojova et al. 2009) that requires further investigation. The ability of *A. castellanii* to uptake CeONP and YtONP also has implications for transmission of microbial diseases. Acanthamoebae are ecologically important as natural grazers that feed on organic and inorganic matter in the soil and water, thereby contributing to the natural recycling of nutrients and minerals. Acanthamoebae also consume bacteria, fungi, and other protists. Therefore, acanthamoebae are important for ecological balance and help regulate microbial populations in nature (Sinclair et al. 1981, Foster and Dormaar 1991, Kreuzer et al. 2006, Khan 2009). The ability of CeONP and YtONP to act as cellular antioxidants, to protect cells, and to elongate their lifespan (Rzagalinski 2005, Schubert et al. 2006, Rzagalinski et al. 2006, Elswaifi et al. 2009) means that these effects may apply to bacteria engulfed by the acanthamoebae. Therefore, environmental contamination by these nanoparticles may lead to the development of bacteria that resist killing in the phagosome and become "superbugs" (Elswaifi et al. 2009) thereby posing a further risk to the environment and to human health. On the other hand, bacteria may be directly exposed to the nanoparticles in the environment, then, if consumed by humans, those bacteria may become more resistant to killing by the immune system or by antibiotics (Elswaifi et al. 2009).

#### CONCLUSIONS

In conclusion, our results demonstrate that CeONP and YtONP can be readily taken up by a protozoan (*A. castellanii*) and a planktonic crustacean (*D. magna*), two integral components in an aquatic food chain. We have also demonstrated that after uptake, CeONP and YtONP are associated with cell and organelle membranes. These findings have implications for the health risks associated with environmental contamination by CeONP and YtONP.

#### AUTHORS' CONTRIBUTIONS

JRP conceived of the study, helped design the study, participated in growing the organisms and exposing them to nanoparticles, participated in interpreting the results, and drafted the manuscript. SFE conceived of the study, helped design the study, participated in interpreting the results, and assisted in drafting the manuscript. CM prepared *Acanthamoeba* for electron microscopy and collected and photographed specimens. GG prepared *Daphnia* for electron microscopy and collected and photographed specimens. All authors read and approved the final manuscript.

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The authors thank Kathy Lowe, Morphology Lab Supervisor, at the Virginia Maryland College of Veterinary Medicine for preparation and examination of samples for electron microscopy. The authors acknowledge the Edward Via College of Osteopathic Medicine for providing funding and laboratory facilities for this project.

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**ABSTRACTS OF PAPERS, 89th Annual Meeting of the  
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University of Richmond, Richmond VA**

**Aeronautical and Aerospace Sciences**

U.S. NAVY AVIATION EVENTS RELATED TO THE HAMPTON ROADS, VA AREA . M. Leroy Spearman, Langley Research Center, Hampton, VA & Robert W. Heath, Canon of Virginia, Newport News, VA. The Navy acknowledged a role for aviation in September 1910 when Capt. Washington I. Chambers was designated as the officer in charge of aviation matters. Soon Glenn Curtiss, a U.S. pioneer in aviation, began meeting with Chambers in an effort to convince the Navy of the value of aircraft for Naval use. In November 1910 Eugene Ely took off in a Curtiss airplane from a wooden platform built over the bow of a ship in the harbor at Hampton Roads, VA. In 1921 the Army Air Service at Langley Field, VA conducted bombing tests off the coast of Virginia in which Army bombers successfully sank a captured German battleship. The tests demonstrated the powerful striking force of air power. The Navy recognized the need for fleet defense and expanded the development of aircraft carriers. In July 1919 the Navy acquired a ship designed to launch and recover airplanes at sea. The ship was commissioned at Norfolk, VA in March 1922 as the USS Langley (CV-1) the Navy's first aircraft carrier. In October 1922, the first take off and landing were made from the CV-1 near Hampton Roads, VA. In November 1922, the first catapult launching was made from the CV-1 located at anchor in the York River near Hampton Roads. In 1933 the first ship designed and constructed as an aircraft carrier, the USS Ranger (CV-4), was launched at the Newport News ship yard. Since then, 28 aircraft carriers have been constructed for the U.S. Navy at that shipyard and Navy aviation was well underway.

SOME NACA/NASA AERODYNAMIC RESEARCH RELATED TO SECURITY AND DEFENSE. M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA . The use of aircraft by European nations in World War I resulted in concern that the U.S. was lagging in aviation developments. This led to an act of the U.S. Congress in 1915 that established the National Advisory Committee for Aeronautics (NACA) with the charge to conduct aerodynamic research. The research began at Langley Field, VA in the early 1920's and over the years this research transformed low-speed, wood and fabric, propeller-driven airplanes into high speed, all-metal, jet and rocket propelled airplanes. This led to the fields of supersonic and hypersonic flight and provided for access to space. The need for space research led to the establishment of the National Aeronautics and Space Administration (NASA) in July 1958. The nucleus of the NASA was the existing NACA with the charge expanded to include space research. The Space Act does specify that the NASA should interact with other agencies on matters affecting security and defense. During the cold war era following World War II, the preponderance of Soviet Union military equipment was perceived as a threat. Accordingly, at NASA-Langley a research activity was undertaken to provide experimental and analytical information to assess a variety of

foreign aircraft and missile concepts of concern to our security and defense. Over the years research information has been acquired for eleven aircraft, twelve surface-to-air missiles, five air-to-air missiles, two air-to-surface missiles, two surface-to-surface missiles, five ballistic missiles, two anti-tank missiles, four reentry vehicles, and one wing-in-ground effect vehicle.

TRACING THE GROWTH OF U.S. AIRPOWER AND THE AIRCRAFT INVOLVED. M. Lerov Spearman, Langley Research Center, Hampton, VA The U.S. military first recognized a role for aviation in 1907 with the establishment of an Aeronautical Division in the U.S. Army Signal Corps. The U.S. Navy acknowledged a role for aviation in 1910. The Army procured an airplane from the Wright Brothers in August 1908 and began flight tests at Ft. Meyers, VA. A year later an improved Wright Flyer was accepted by the Army as 'Airplane No. 1'. Another American designer, Glenn Curtiss, was becoming well known and in 1911 a Curtiss airplane was the second airplane to be sold to the U.S. Army. The first airplane to be built for the U.S. Navy was a Curtiss airplane 1911. The first appropriation for military aeronautics was in the 1912 War Department for \$125,000. From these funds, orders were placed for three Wright airplanes and two Curtiss airplanes. War broke out in Europe in 1914 and it was apparent that a disparity existed between European progress and U.S. foot-dragging in the field of military aviation. In 1915 the U.S. Congress approved legislation to create a research facility to enhance aeronautical programs. In 1916 property was purchased just north of Hampton, VA that provided a flying field for aeronautical research and flight testing. Named Langley Field, a portion of the field was set aside for construction of the newly created National Advisory Committee for Aeronautics (NACA). The U.S. entered World War I in 1917 and did learn from flying and building European airplanes. Significant growth began in the 1920's with trainers, bombers, first Navy fighters and Army pursuits, observation airplanes, the first monoplanes, attack airplanes, first jet aircraft in early 1940's, first twin-engine fighter jets, swept-wing concepts, advanced supersonic designs such as the Century series of fighters - a total of over 60 military aircraft designs appeared from 1940 to 1980. Currently some aircraft using stealth technology are being deployed.

### **Agriculture, Forestry and Aquaculture Science**

YIELD AND RIPENING OF GREENHOUSE TOMATOES IN RESPONSE TO DAYTIME TEMPERATURE. Mark Kraemer & Françoise Favi, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. Greenhouse-grown tomatoes are a high-value crop for limited-resource producers in the mid-Atlantic states. Increased heating costs in recent years have encouraged many to reduce growing temperatures. Measurement of photosynthetic rates indicated lower temperatures were feasible and some professional growers recommended them. However, little actual data was available in the published literature. Thus, we evaluated tomato yield differences between a warm and cool greenhouse sections. The average daytime temperature in the warm section averaged about 2°C greater than the cool greenhouse. Night temperatures were similar. Each section had 18 tomato plants (var. Trust) arranged in three rows.



The experiment was run for 3 months in the fall of 2009 and again in 2010, with warm and cool sections reversed. The results indicated that total fruit yield was the same for both sections but that fruit ripened faster in the warm section. It is recommended that growers allow temperatures to rise during sunny days by reducing venting and using CO<sub>2</sub> generators. Increased heating costs during cold, sunny periods could be repaid with earlier harvests.

LOCATION OF THRIPS (THYSANOPTERA: THIRIPIDAE) ON SOYBEAN SEEDLINGS AND IMPLICATIONS FOR SAMPLING. J.A. Samler<sup>1</sup>, D.A. Herbert<sup>1</sup>, S. Malone<sup>1</sup>, D. Owens<sup>1</sup>, T.P. Kuhar<sup>2</sup>, & C. Brewster<sup>2</sup>. <sup>1</sup>Department of Entomology, Virginia Tech Tidewater Agricultural Research and Extension Center, Suffolk, VA 23437. <sup>2</sup>Department of Entomology, Virginia Tech, Blacksburg, VA 24061. Thrips feeding can cause significant injury to soybean seedlings resulting in stunted growth and delayed maturity. Determining accurate numbers of thrips on plants is necessary for making control decisions. Currently, sampling is based on assessing populations on whole plants which very labor intensive. This research was conducted to determine where thrips are located on soybean plants and whether subsamples of plant material are adequate for monitoring populations. Using a stratified random sampling method, soybean plants were randomly selected and systematically sectioned into three parts: the top three unopened leaflets (Terminal); the first fully expanded trifoliate (Trifoliate); and the remainder (Remainder) of the plant which was cut at the base just above the soil surface. Thrips larvae and adults were washed from each plant sample and the leaf area was measured. Results showed that the soybean Terminal has the highest density of thrips larvae ( $\mu = 1.412$ ) while the Remainder of the plant has the highest absolute count ( $\mu = 4.515$ ) when density is not a factor. There was no correlation between the number of larvae and leaf area in any of the plant sections (Terminal:  $r = -0.0086$ ,  $p = 0.9628$ ; Trifoliate:  $r = -0.2742$ ,  $p = 0.1289$ ; Remainder:  $r = -0.1158$ ,  $p = 0.5280$ ). Larval counts from the Remainder of the plant had the strongest correlation with whole plant counts ( $r = 0.895$ ,  $p = <0.0001$ ). Implications are that whole plant samples are the most accurate even though labor intensive.

INVESTIGATING THE ROLE OF OLFACTION IN THE HOST PLANT SELECTION OF HARLEQUIN BUG (MURGANTIA HISTRIONICA), PEST OF COLE CROPS. Wallingford, A.K., T.P. Kuhar and P.B. Schultz. Dept. of Entomology, Virginia Tech. Harlequin bug (HB) is a piercing-sucking pest of cole crops. Adult and nymphal feeding causes cosmetic injury to vegetables sold as greens and, if left untreated, HB infestations can stunt or kill a plant. There is potential for managing HB by planting a preferred or more attractive host plant, or trap crop, to divert insect feeding away from the protected cash crop. This study evaluated several species of brassicales and a non-host control for HB host preference and thereby the suitability for use as a trap crop. Caged choice tests show that HB adults prefer to feed on mustard (*Brassica juncea* 'Southern Giant Curled') over, arugula (*Eruca sativa*), bean (*Phaseolus vulgaris*), collard (*B. oleracea* 'Champion'), rapini (*B. rapa* 'Roquette') and rapeseed (*B. napus* 'Athena'). Olfactometer choice tests showed that male HB are attracted to the odors from the leaves of both bean (non-host) and mustard (preferred host) over clean air, but when given a choice

between the two, males orient to mustard over bean. This is not the case for females as they do not orient to odors from mustard or bean, and show no preference for the odors of one over the other. Male and female HB both orient to odors produced by the combination of odors from males feeding on mustard leaves (presumably emitting an aggregation pheromone) over mustard leaves alone, while neither orient to males feeding on bean versus bean alone and do not orient to males alone versus clean air. Implications for trap crop implementation are discussed.

**POTENTIAL USE OF CHLOROPHYLL FLUORESCENCE TO IDENTIFY DIFFERENCES IN TRANSPIRATION EFFICIENCY IN PEANUT.** Jacqueline F. Hawkins & George T. Byrd, School of Natural Sciences & Mathematics, Ferrum College, Ferrum, VA 24008. Production of peanut (*Arachis hypogaea* L.) often occurs in areas prone to drought, therefore genotypic selection should be based on efficient use of water. This study examined the potential use of chlorophyll fluorescence for assessing genotypic variation in peanut transpiration efficiency (TE), defined as the weight of dry matter per unit of water transpired. The experimental hypothesis was the rate of decline in chlorophyll fluorescence in peanut leaves would differ under different watering regimes and among genotypes and these differences would be correlated with TE. Since reports show TE to be correlated with specific leaf weight (SLW), this trait was also assessed. Seventeen genotypes were grown in closed containers in a soil-sand mix and subjected to two soil moisture regimes –well-watered and water-deficit. Peanut genotypes differed in TE, however, TE did not differ between well-watered and water-deficit conditions. Transpiration efficiency was positively correlated with SLW across water treatments ( $r = 0.75$ ). Correlation between TE and chlorophyll fluorescence, however, was not significant. Although chlorophyll fluorescence has shown promise to indicate water-deficit stress responses in species like cotton and sorghum, peanuts showed little difference in the decline in chlorophyll fluorescence under well-watered and water-deficit conditions. Further research is needed to determine the relationship between chlorophyll fluorescence and traits associated with drought tolerance in peanut. (Supported by a Faculty Development Grant, Ferrum College).

**POTENTIAL FISH PATHOGENS FOR CAGE AQUACULTURE IN VIRGINIA.** David Crosby, Virginia Cooperative Extension, Virginia State University, PO Box 9081, Petersburg, VA 23806. Many farm pond owners raise catfish in cages for personnel consumption or to sell as a farm commodity to local markets in Virginia. These farmers typically buy catfish fingerlings that are sold into Virginia from out of state commercial catfish fingerling farms. What fish farmers do not realize that there is a potential disease risk involved with caged catfish. The typical bacterial pathogens that cage producers would encounter are *Aeromonas* and *Columnaris*. These are quite common in the aquatic environment and one of the leading causes of disease problems in cages. A new strain of *Aeromonas hydrophila* that has originated in Alabama is highly pathogenic. This strain has been observed in Virginia. A fish health study conducted at VSU during 2007 to 2009 found that catfish fingerling from commercial farms had various external parasites. These catfish typically had *Trichodina*, proliferative gill disease and *Ligistaluriadus* on the gills. In one case, highly pathogenic bacteria, Enteric Septicemia of Catfish, which is common to

catfish farming, was found during the study. This study also found that fish were exposed to endemic pathogens such as Ich and Trichophyra. By stocking commercial produced catfish a farmer increases the risk of bringing in pathogens that would cause a disease outbreak, or result in an epizootic from an endemic pathogen(s), or worst case scenario introduce a new potential pathogen to the fish farm.

THE WHY, WHERE, AND WHEN OF CEREAL LEAF BEETLE (*OULEMA MELANOPUS* L.). Chris Philips<sup>1</sup>, Ames Herbert<sup>1</sup>, Tom Kuhar<sup>1</sup>, Dominic Reisig<sup>2</sup>, and Sean Malone<sup>1</sup>. <sup>1</sup> Department of Entomology, Virginia Tech, Blacksburg, VA. and <sup>2</sup> Department of Entomology, North Carolina State University, Plymouth, NC. Because of the difficulty in predicting when and where cereal leaf beetle outbreaks will occur many growers in the Southeast apply insecticides on a calendar basis rather than using a threshold-based IPM approach. Our challenge is to develop new information and procedures that will encourage growers to reevaluate the way they are approaching spring-time insect control in wheat, and consider adoption of the IPM approach. Using small plot trials in 2010 and 2011, we found that a number of insecticides were effective at controlling CLB when applied at the right time. We also found that an IPM approach was more effective at controlling CLB than an automatic application. In addition, large scale surveys indicated that CLB was not randomly distributed on a field and regional scale. Finally, degree day information was used to create a predictive model for when peak CLB egg laying will occur. This model was used to predict the average calendar date of peak eggs for each population as well as for the average of all populations. In 2010 and 2011, the model predicted the average egg peak within 2 days and 1 day, respectively. Larval peaks occurred approximately 16 days after egg peak in 2010 and 17 days after egg peak in 2011. Given the accuracy of 182 DD, historical weather information was used to create a predictive map of when areas will exceed 182DD. This map predicted the calendar date for each area in Virginia within 4 days.

OPTIMAL GROWTH CONDITIONS ACROSS ORGANIC Highbush BLUEBERRY CULTIVARS AND SOIL TREATMENTS, Braydon P. Hoover, Denay M. Fuglie, & Roman J. Miller, Eastern Mennonite Univ., Harrisonburg, VA 22802. Four growth parameters including height, primary stalk diameter, relative 'bushiness', and volume of plant cylinder were determined for four soil treatments and five highbush blueberry cultivars at Knoll Acres Organic Blueberry Farm in the autumn of 2010. Planter's Choice and pine straw composts had significantly higher values than both horse and sheep manure in height ( $75.5 \pm 2.7$ ) and bushiness ( $1171 \pm 140 \text{ cm}^3$ ), stalk diameters ( $8.20 \pm 1.60 \text{ cm}$ ) and plant cylinder volumes ( $381 \pm 37 \text{ dm}^3$ ) respectively. Chandler had significantly higher values in both the stalk diameter ( $8 \pm 0.36 \text{ mm}$ ) and bushiness ( $976.38 \pm 172.8 \text{ cm}^3$ ) parameters. Duke bushes had significantly lower plant cylinder volume ( $114683 \pm 14639 \text{ cm}^3$ ) than all other cultivars and Bluecrop bushes had significantly greater average heights ( $77.85 \pm 2.6 \text{ cm}$ ). The photosynthetic rates of Jersey bushes were found to be significantly higher than Bluecrop, however, no significant difference was found to indicate that soil treatments affected photosynthetic rate.

EFFICACY OF A NATIVE BEE FOR POLLINATION OF EASTERN ORCHARDS. Melanie Cutter & Mark Kraemer, Agricultural Research Station, Virginia State Univ., Petersburg, VA 23806. The blue orchard bee, *Osmia lignaria* Say, has been shown to be an excellent pollinator of tree fruits in western North America. A subspecies of this bee is widespread in eastern North America and may be similarly effective. The objective of our study was to determine pollen preference of this bee with respect to nest placement. Sets of 4 nest shelters were placed within the orchard, an adjacent hardwood forest, and along the border between orchard and forest. Each shelter contained a wood block with 24 nest holes. Prior to apple bloom, each nest shelter was provided with 3 nests of adult bees that were ready to emerge. Weekly images of nests were taken to date the completion of new nests. Pollen samples were collected from individual nest cells in early May, after the end of apple bloom. Random samples of 300 pollen grains per bee nest cell were identified to species using a scanning electron microscope. The results indicated that the bees preferred to collect pollen from nearby Eastern redbud (*Cercis canadensis* L.) trees than the orchard fruit trees. Placement of nest shelters did not make a significant difference in pollen preference. We concluded that eastern redbud trees near orchards could reduce the pollination efficacy of *O. lignaria* within eastern orchards.

### Astronomy, Mathematics and Physics with Materials Science

OPTICAL CHARACTERIZATION OF SELF-ASSEMBLED POLYMER/MICROPARTICLES THIN FILMS. Daniela M. Topasna & Gregory A. Topasna, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. Thin films are an important component in many optical and electrical devices, including optical filters. We fabricated multiple layer thin films of sodium salt of poly(styrene sulfonate)/titania using a layer-by-layer self-assembly method. The optical properties of these films were determined based on the calculations of a theoretical numerical model that we developed. The UV-VIS-NIR measurements of these films performed in the 300-2400 nm range confirmed the relationship between the type and number of layers and were consistent with the modeled transmittance values at specific wavelengths.

DETERMINATION OF PHOTON ARRIVAL RATES AND S/N RELATIONSHIPS FOR THE VIRGINIA MILITARY INSTITUTE'S 0.5 METER TELESCOPE. Gregory A. Topasna & Daniela M. Topasna, Virginia Military Institute, Lexington, VA 24450. We determined the photon arrival rate for unfiltered CCD images using the Virginia Military Institute's 0.5 meter telescope. Observations of the open clusters NGC 1502 and M 44 were made using an Alta U6 CCD camera with different integration times. Aperture photometry was performed on stars of known visual magnitude and, using the standard CCD equation, the signal-to-noise ratio was determined for the different integration times. The photon arrival rate for each star was then determined from these data and the CCD equation. As a function of magnitude  $m$  the photon arrival rate is approximately equal to  $10^{(9.26-m/2.4)}$ . The

airmasses of the cluster images differed by  $\sim 1$  and our analysis showed that arrival rates were not strongly dependent on this airmass difference.

LOVE WAVE PROPAGATION IN A POROUS ELASTIC LAYER UNDER RIGID BOUNDARY. Shishir Gupta & Dinesh K. Majhi, Dept. of Applied Mathematics, Indian School of Mines, Dhanbad-826 004, India. In this paper, propagation of Love waves in a porous elastic layer under a rigid boundary over a porous elastic half space has been considered. Pores contain nothing of mechanical or energetic significance. The study shows that such a medium transmits two types of Love waves. The first front depends upon the change in volume fraction of the pores. The second front depends upon the modulus of rigidity of the elastic matrix of the medium and is the same as the Love wave in an elastic layer under a rigid boundary over an elastic half-space. It is found that the phase velocity of Love waves is considerably influenced by porosity. It is also observed that the first wave front is faster than that of shear wave in the porous medium due to change in volume fraction of the pores.

THERMALLY DRIVEN ATMOSPHERIC ESCAPE: MONTE CARLO SIMULATIONS FOR TITAN'S MULTI-COMPONENT ATMOSPHERE. Orenthal J. Tucker & Robert E. Johnson, University of Virginia, Charlottesville, VA., 22904. Kinetic Monte Carlo model simulations of thermal escape from Titan's upper atmosphere are used to reproduce the Cassini INMS density measurements of  $N_2$  and  $CH_4$  without requiring significant loss rates as proposed by recent continuum models. In the kinetic model intermolecular collisions between  $N_2$ ,  $CH_4$  and  $H_2$  are described using temperature dependent cross sections and allowing the exchange of internal energy as well as translational between molecules. Fits to INMS density data for  $N_2$  obtained when Titan was in Saturn's plasma sheet indicate  $H_2$  has a Jeans parameter  $\sim 3$  at the exobase. The kinetic Monte Carlo simulations obtain an  $H_2$  loss rate  $\sim 1 \times 10^{28} H_2/s$  which is essentially the Jeans rate. The results from multi-component kinetic simulations are used to examine the change in the  $H_2$  escape rate when Titan is not in the plasma sheet to when it is in the plasma sheet using average magnetospheric conditions to obtain the energy deposition by the flux to  $O^+$  and  $H^+$  into Titan's upper atmosphere.

THE MEANING OF EINSTEIN'S EQUATION. Emory F. Bunn, Physics Department, University of Richmond, Richmond, VA 23173. The Einstein field equation, relating spacetime curvature to the matter stress-energy tensor, is one of the fundamental laws of physics, but, due to the level of mathematical machinery required to express it, it is generally omitted from the undergraduate curriculum. However, it is possible to express the entire content of this equation in relatively simple geometric terms, without the apparatus of tensor calculus. To be specific, the Einstein equation relates the rate of change of the volume of a small ball of initially-comoving test particles to the density and pressure of matter in the ball's interior.

THE COSMIC MICROWAVE BACKGROUND RADIATION. Emory F. Bunn, Physics Department, University of Richmond, Richmond, VA 23173. Observations of the cosmic microwave background radiation are among the chief causes of the rapid advance in our understanding of cosmology over the past 20 years. Microwave background temperature anisotropy measurements have confirmed the presence of acoustic waves in the early Universe, providing support for the standard gravitational instability paradigm and allowing us to deduce the properties of acoustic waves in the early Universe and hence to measure cosmological parameters. Attention is now shifting to measurements of microwave background polarization. Polarization measurements can shed light on several aspects of cosmology, but arguably the most exciting is the prospect of detecting “B modes,” which could provide direct evidence of an inflationary epoch. Although existing data are in most ways remarkably consistent with the standard cosmological model, some apparent anomalies have been noted on large scales. The significance of these anomalies is hotly debated. To settle the question, we need a new data set that probes perturbations on similar scales but is statistically independent of the existing measurements. Future microwave background polarization maps may provide such a data set.

ORBITS AND SCALING FOR AN ISOTROPIC METRIC. J. D. Rudmin, Dept. of Integrated Sci. and Tech., James Madison University, Harrisonburg VA 22807. Scaling of physical quantities shows the symmetries of an isotropic metric, including extension to the equivalence principle. For example, invariance of Planck's constant under gravitational scaling provides consistency of general relativity with quantum mechanics. Invariance of charge provides consistency with electromagnetism. Transitivity of scaling eliminates the traditional need for a globally preferred reference frame. Rather, diagonalization of the metric yields local rest frames. Scattering, orbital period, and precession offer ways to distinguish an isotropic from a Schwarzschild metric.

AN ANNUAL PATTERN IN WATER TEMPERATURE DIFFERENTIALS BETWEEN ADJACENT REGIONS OF THE CHESAPEAKE BAY, VIRGINIA. T.C. Mosca III, Dept. of Mathematics, Rappahannock Community College, and W. C. Coles, Div. of Fish and Wildlife – Dept. of Planning and Nat. Res. Each winter the Atlantic Ocean, lower Chesapeake Bay, and the major tributaries cool to about the same temperature. In the spring, water farther from the ocean warms faster, and the difference in temperature between the bodies grows. The maximum difference occurs in spring near the ocean, in summer farther upstream. Warming occurs by the influence of solar radiation at the surface, the rate of which is the same in all locations. The ocean simply has more water per unit of surface area, and warms more slowly. The lower regions of Chesapeake Bay and the rivers will warm more slowly than the upper regions because ocean water circulates in these parcels. The lag in warming is attributable to the exchange of energy with the Atlantic Ocean, even at the farthest distances examined in this study. The different timing of the lags in the maximum temperature differences that occur in the upper reaches (June vs. April) is an artifact of distance from the ocean. The only area where this pattern changes is in the lower portion of the Rappahannock River, probably due to a ledge across the mouth of the river, where depths quickly become

half that of the nearby deep zone. We examined the lags as differences in long-term average monthly temperatures of regions of the Bay and tributaries. This feature of energy transfer is probably present in other estuaries, because a defining feature of an estuary is density driven circulation.

EFFECTS OF THE CORIOLIS FORCE ON THE LARGE-SCALE ATMOSPHERIC TURBULENCE. Iordanka N. Panayotova, Department of Mathematics and Statistics, 4700 Elkhorn Ave., Old Dominion University, Norfolk, VA 23529. Earth's rotation causes an inertial force, known as the Coriolis force. The Coriolis force is small but its effects become noticeable for motions occurring over large distances and long periods of time (large-scale motions). Well known examples of large-scale formations in the atmosphere are cyclonic/anticyclonic systems, or jet-streams and atmospheric waves. On the other hand the large-scale geostrophic turbulence is a chaotic three-dimensional nonlinear motion of the fluids that are near to the state of geostrophic and hydrostatic balance. A simple three-dimensional model of the tropopause dynamics is a model with introduced a quasi-horizontal interface separating regions of homogeneous potential vorticity of different values. The quasi-geostrophic (QG) approximation to this model reduces dynamics of the flow to quasi-two-dimensional turbulence. In this way, the three-dimensional flow is entirely modeled by the horizontal advection of potential temperature on the interface (surface quasi-geostrophic (sQG) model). Including weakly non-linear dynamics and the meridional variation in the Coriolis parameter ( $\beta$ -effect) will result in the so called  $\beta$ -sQG+1 numerical model. Here this model is applied to study the effects of the Earth's rotation on the large-scale atmospheric turbulence. The numerical simulations capture some important dynamical characteristics of the large-scale atmospheric dynamics known from observations, in particular the meridional asymmetries in eddies spatial and time scales, and their orientation.

UNCERTAIN GROUND: MAPPING ERRORS THROUGH THE POM-SAT MODEL OF PALEOCLIMATE RECONSTRUCTION. Marshall G. Bartlett, Dept. of Physics, Hollins University, Roanoke, VA 24020. Borehole temperature-depth profiles contain information about ground surface temperatures (GST) a region has experienced in the past and provide complementary information to the surface air temperature (SAT) record of climate change. The borehole method of climate reconstruction assumes the dominant heat transport mechanism in the upper few hundred meters of the earth's crust is conduction; mathematically, conduction is a compressive (information losing) mapping. Consequently, the solution obtained is non-unique. One robust means of dealing with the non-uniqueness problem is to limit the number of parameters sought in the solution space. However, even when only a single parameter (the pre-observation mean GST, or POM) is sought in the inversion, a certain amount of a priori information must be assumed. I am interested in how uncertainties in this a priori information are mapped into uncertainties in the solution space. I perform a Monte Carlo analysis to investigate how uncertainties in our model of the ground thermal regime, SAT observations, and the background geothermal gradient are mapped into the solution space of the POM-SAT method of

climate reconstruction from borehole data. Results indicate that uncertainties in the SAT time series and thermal parameterization of the ground are reduced by an order of magnitude, while uncertainties in the geotherm are magnified by an order of magnitude in the solution-space.

### **Biology** **with Microbiology and Molecular Biology**

THE EFFECTS OF BACTERIOPHAGE INFECTION ON *BACILLUS ANTHRACIS* DELTA STERNE AND *BACILLUS THURINGIENSIS*. Catherine A. Johnson & Lynn O. Lewis, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. *Bacillus anthracis* and *Bacillus thuringiensis* are soil dwelling bacteria that are capable of forming endospores. These bacteria go through the processes of sporulation and germination to form the endospores and vegetative cells. Both species of bacillus can be found in various soil samples, along with species of bacteriophages that target each bacterium. The goal of this research was to isolate one temperate bacteriophage for *Bacillus anthracis* Delta Sterne (Delta Sterne) and one temperate bacteriophage for *Bacillus thuringiensis* Al Hakam (Al Hakam) and then determine the effects the bacteriophages have on the sporulation and germination processes for Delta Sterne. A temperate bacteriophage was isolated for each bacillus species, with the one that infects Delta Sterne being called Texas 1 4CI. It was able to cross infect Al Hakam, but the Al Hakam temperate phage was unable to cross infect Delta Sterne. There were no apparent morphological differences between colonies of infected Delta Sterne and uninfected Delta Sterne through sporulation and germination. However, it appears that with successive sporulation and germination, the infectivity of the bacteriophage for Delta Sterne increases from 1 plaque at  $10^{-3}$  dilution, to 32 plaques at  $10^{-3}$  after a second sporulation and germination. (Supported by UMW Undergraduate Student Research Grant.)

CHARACTERIZATION OF MYCOBACTERIOPHAGE VENKMAN. Thien T. Phan & Lynn O. Lewis, Department of Biological Sciences, University of Mary Washington, Fredericksburg, VA 22401. Bacteriophage are abundant in the environment and have co-evolved with the bacteria they target. Through soil collected, we were able to have a better understanding of how mycobacteriophage interact with their host, *Mycobacterium smegmatis*. One bacteriophage (Venkman) was chosen for genomic sequencing. Restriction enzyme digests were performed to compare the phage's DNA to other known bacteriophage DNA. Electron microscopy was performed to examine the morphology of the Mycobacteriophage. By using different bioinformatics programs, we determined that the Mycobacteriophage Venkman is in the F cluster and within the F1 subcluster. The genome size is approximately 50 kbp – to 60 kbp, but is unfinished at this time. Further investigations are still being performed on the genome of Mycobacteriophage Venkman. (Supported by UMW Undergraduate Student Research Grant.)



POST TRANSLATIONAL MODIFICATION OF A BACTERIAL PROTEIN IN HUMAN CELLS. Moushimi Amaya, Ancha Baranova & Monique van Hoek, Department of Systems Biology, George Mason University, 10900 University Blvd., Manassas, VA 20110. This study is an on-going investigation into the post translational modification, the prenylation, of a *Francisella tularensis* protein in human cells. Prenylation is a lipid modification, whereby either a farnesyl or geranylgeranyl group is added to a nascent protein harboring a C-terminal CAAX motif, which results in subsequent targeting of the mature protein to the cell membrane. *F.tularensis* is a gram negative bacterium that causes the disease tularemia and can potentially be used a bioweapon due to its low infectivity dose (10-15 organisms) upon inhalation. Bioinformatical analysis using the Prenylation Prediction Suite program revealed a single *F.tularensis* protein that could potentially undergo prenylation. The proposed prenylated protein is 13kDa in size and is of unknown function. Human alveolar lung epithelial cells (A549) are the model system chosen to test for prenylation of the *Francisella* protein. Preliminary Western blot experiments have confirmed that the protein of interest is expressed in A549 cells. We aim to determine if prenylation does indeed occur in A549 cells. We further aim to investigate, by use of inhibitors, which type of prenylation is undertaken: farnesylation or geranylgeranylation. We also aim to determine the localization of the potentially prenylated *Francisella* protein in A549 cells by employing confocal microscopy analysis.

FUNCTIONAL GEMOMIC ANALYSES REVEAL COMPLEX TRANSCRIPTIONAL REGULATORY NETWORKS MEDIATING DENDRITIC ARCHITECTURE. Eswar Prasad R. Iyer<sup>1,2</sup>, Srividya Chandramouli Iyer<sup>1,2</sup>, Ramakrishna Meduri<sup>1,2</sup>, Dennis Wang<sup>1,2,3</sup>, and Daniel N. Cox<sup>1,2,4</sup> School of Systems Biology, George Mason University, Manassas, VA 20120, USA,<sup>2</sup>Krasnow Institute for Advanced Study, George Mason University, Fairfax, VA 22030, USA, <sup>3</sup>Present Address: Yale University, New Haven, CT 06520, USA Elucidating the molecular mechanisms controlling dendrite development is key to understanding the pivotal role these structures play in influencing synaptic integration and neural function. Despite significant advances in this field, genetic pleiotropy remains a significant impediment to investigating such complex developmental processes. To circumvent this problem, we have applied class specific neuron transcriptional expression profiling coupled to an *in vivo* RNAi functional validation screen in order to dissect the molecular bases of *Drosophila* class IV dendritic arborization (da) neuron dendritogenesis. Microarray analyses reveal transcriptional regulation as one highly enriched biological and functional category with 420 transcription factors significantly expressed in class IV neurons. Among these, we identify roles for 268 genes in mediating a broad spectrum of functions including dendritic field coverage, branching, routing, and tiling. Collectively, our analyses provide a more comprehensive framework of the role complex transcriptional networks play in directing distinct aspects of class specific dendrite morphogenesis.

CUT MEDIATED TRANSCRIPTIONAL REGULATION OF THE COPII SECRETORY PATHWAY DIRECTS CLASS SPECIFIC DENDRITE MORPHOGENESIS IN *DROSOPHILA*. Srividya C. Iyer, Eswar P.R. Iyer,

Ramakrishna Meduri, Madhu Karamsetty, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Elucidating the molecular mechanisms controlling dendrite development is key understanding how neuronal morphologies arise and how they function in achieving synaptic integration and neuronal function. Recent studies demonstrate select secretory pathway genes act in preferentially affecting dendritic growth. Phenotypic analyses of *sec31* mutants reveal a reduction in dendritic branching implicating the COPII secretory pathway in regulating dendritic complexity. Furthermore, gain-of-function (GOF) analyses indicate *sec31* differentially effects dendritic complexity in distinct da neuron subclasses. Microarray analyses, quantitative RT-PCR and immunohistochemistry experiments reveal that overexpression of the homeodomain transcription factor Cut upregulated expression levels of the COPII-mediated secretory pathway genes as well as another key transcription factor, CrebA. Moreover, simultaneous expression of Cut coupled with RNAi knockdown of *CrebA* suppressed the Cut GOF phenotype indicating that CrebA functions as a downstream effector of Cut mediated transcriptional regulation in da neurons. Consistent with this regulatory relationship, overexpression of CrebA in da neurons likewise leads to higher expression levels of components of ER-to-Golgi transport. Collectively, these findings provide novel insight into the role of transcriptional regulation of the COPII-mediated secretory pathway in mediating class specific dendrite morphogenesis.

NEUROPEPTIDE AF-INDUCED ANOREXIA IS ASSOCIATED WITH CHANGES IN HYPOTHALAMIC CHEMISTRY IN SPRAGUE-DAWLEY RATS. Brandon A. Newmyer and Mark A. Cline. Dept of Biol, Radford University, Radford VA 24142. We recently demonstrated that NPAF's anorectic effect is associated with changes in hypothalamic chemistry in nuclei associated with satiety perception in Cobb-500 chicks. In order to elucidate whether this effect was conserved through divergent evolution, NPAF was centrally administered to Sprague-Dawley rats and food intake as well as NPAF-associated changes in brain chemistry were observed. NPAF reduced food intake in rats at similar doses and magnitudes as it did in chicks and also affected hypothalamic chemistry. Similar to chicks, central NPAF was associated with increased neuronal activation in the magnocellular region of the paraventricular nucleus. These data support that NPAF's anorectic effect is conserved in a mammalian model and thus may be a logical target utilize in the treatment of human eating disorders.

EFFECTS OF COMBINED VITAMIN C & E TREATMENT ON PLAQUE FORMATION IN ALZHEIMER'S DISEASE. Anum K. Shaikh & Deborah A. O'Dell, Dept. of Biol., University of Mary Washington, Fredericksburg VA 22401. Alzheimer's disease (AD) is characterized by the inflammation and  $\beta$ -amyloid plaques in the brain. The improper cleavage of amyloid precursor protein (APP) which leads to  $\beta$  amyloid plaques may result from inflammation. We studied the effects of these Vitamins E and C on inflammation and plaque formation in a transgenic mouse model of AD. Mice were divided into three experimental groups that received Vitamin C, Vitamin E or both Vitamin C and E for a period of 34 weeks beginning at 14 weeks of age. Levels of tumor necrosis factor alpha (TNF- $\alpha$ ) and

$\beta$ -amyloid in homogenized brain preparations were measured using a direct ELISA method. The number and size of the plaques in sections of formalin fixed and paraffin embedded cerebral cortex was compared between the groups. The levels of  $\beta$ -amyloid were marginally significantly lower in the combined Vitamin C and E treatment group compared to the control ( $p=0.056$ ). Vitamin E treatment alone did not lower  $\beta$ -amyloid relative to the control. Vitamin treatments also show reduced levels of inflammation as measured by levels of TNF- $\alpha$  compared to the control with Vitamin C and Vitamin C&E showing the lowest levels. There was no significant difference noted in comparison of the number and size of plaques between all the groups. These results show that Vitamin C and E may afford the greatest protective effects against AD. (Supported by: Chi Beta Phi Honorary Society, The Virginia Academy of Science, Univ. of Mary Washington Undergrad Research Funds).

A SYSTEM GENOMICS APPROACH FINDS CANDIDATE GENES FOR NON-INSULIN DEPENDENT DIABETES MELLITUS. Lataisia Jones & Glenn C. Harris, Dept of Biology, Virginia State University, VA 23806. Non-insulin dependent diabetes mellitus (NIDDM) is one of the most significant chronic human diseases, affecting over 20 million people in the United States (7% of the population). NIDDM is associated with obesity and characterized primarily by insulin resistance and impaired insulin production. Evidence has been building that NIDDM is a multifactorial complex disease, with many different genes and gene-gene and gene-environment interactions potentially contributing to the physiological symptoms. Here we demonstrate a systems genomics approach to finding likely candidates for NIDDM. A review of linkage analysis studies found a region on human chromosome 01 significantly associated with NIDDM symptoms. Syntenic regions in rats and mice were identified and subjected to a single nucleotide polymorphism (SNiP) analysis. Only one gene was found in the mouse and rat syntenic regions that exhibited functional SNiPs in symptomatic models. A functional SNiP was defined as producing an amino acid change in the resulting protein. The gene, *Tchhl1*, has not been previously implicated as a candidate gene for NIDDM or obesity. Future projects are described that will attempt to characterize the function of *Tchhl1* in NIDDM phenotypes.

CHROMATIN STATE MAPPING IN NEUROBLASTOMA CELLS IDENTIFIES GENES POTENTIALLY IMPORTANT FOR MAINTAINING PLURIPOTENCY. Shaili Shah & Melissa A. Henriksen, Dept. of Biol., University of Virginia, Charlottesville VA 22903. Neuroblastoma is a common childhood cancer that arises from the neural crest. The clinical diversity seen in neuroblastoma is due to its cellular heterogeneity. The Intermediate (I-type), highly malignant, cell differentiates into both a non-malignant (S-type) cell and a less malignant Neuroblastic (N-type) cell. The multipotent I-type cell has been identified as a cancer stem cell due to its ability to self-renew and differentiate. Since epigenetic mechanisms underlie the phenotypic differences among these three cell types, chromatin state mapping was used to identify potential bivalent genes involved in cancer stem cell multipotency and differentiation. Of the 6 genes identified, one gene, *MSX2*, was examined further due to its known role in triggering apoptosis during neural crest development. Since this gene was not expressed in I-type cells, the potentially inappropriate silencing of

this gene was reversed by over-expression in I-type cells. Ongoing experiments focus on using inducible lentiviral based methods to infect the cancer stem cell and quantify cell death in the cancer stem cell in comparison to the other, differentiated, neuroblastoma cells. These preliminary over-expression experiments suggest that MSX2 may also trigger apoptosis of the cancer stem cell. The aberrant chromatin pattern at MSX2's promoter may cause inappropriate gene silencing, and future experiments will determine how crucial this gene is for maintenance of the neuroblastoma cancer stem cell. Understanding the epigenetic mechanisms that govern genes implicated in cancer stem cell maintenance may offer novel therapeutic targets for cancer treatment.

MELANOMA-ASSOCIATED SUPPRESSION OF DENDRITIC CELL MATURATION/ACTIVATION DEPENDS ON TUMORIGENICITY. Kristian M. Hargadon, Osric A. Forrest, & Pranay R. Reddy, Dept. of Biol., Hampden-Sydney College, Hampden-Sydney VA 23943. The accumulation of data demonstrating immunity to cancer over the last two decades has sparked significant interest in the field of tumor immunology. However, while the existence of anti-tumor immunity is promising, tumor cells have frequently been reported to induce anti-tumor immune dysfunction. Despite the overwhelming significance of this problem, the basis for this immune dysfunction is often poorly understood. Dendritic cells play critical roles in both innate and adaptive immunity, and their numerous functions are tightly linked to their maturation and activation status. Here, we characterize the murine dendritic cell line DC2.4 as a model for studying dendritic cell maturation and activation, and we evaluate the influence of melanoma tumor cells on these processes. Exposure of DC2.4 cells to the Toll-like receptor ligand lipopolysaccharide induces both maturation and activation of these cells, characterized by upregulation of costimulatory molecule expression and proinflammatory cytokine/chemokine production. This maturation and activation is suppressed by soluble factors derived from both the highly tumorigenic B16-F1 and the poorly tumorigenic D5.1G4 murine melanoma cell lines. Interestingly, the extent of DC2.4 immunosuppression by these melanomas correlates with their tumorigenicity, suggesting a vital role for dendritic cell/tumor cell interactions in the regulation of anti-tumor immunity and tumor outgrowth.

PATTERNS OF TRANSIENCY, SEX BIAS, AND BODY WEIGHT IN OPEN-HABITAT RODENT POPULATIONS. Stephen E. Rice & Robert K. Rose. Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529-0266. As the most numerous and diverse order of the Class Mammalia, rodents often have served as a common basis for study and modeling biological concepts. Dating from the writings of Robert Collett in the late 19th Century, rodents are believed to live their lives within a small area, with juvenile males the most likely to disperse. To test these assumptions we examined long-term capture-mark-release data from grid populations with attention to the weights at first capture and the transiency/residency patterns of herbivorous rodents studied in old-fields in Virginia, Illinois, and Kansas. Although few studies report the proportions or sexes of juveniles among first-captured animals, many studies have indicated that nearly half of tagged rodents are never seen again. In order to

evaluate the evidence that different species of rodents are philopatric, we examined the sex ratios and body weights of newly tagged old-field rodents and their patterns of transience or residency. Transience was associated with season for Virginia and Kansas cotton rats. Both populations had significant proportions of adults and displayed an association of population groups. Animals in breeding condition were male-biased for Virginia and Kansas. The breeding individuals in Virginia were independent of residency status, whereas Kansas breeders were associated with a high level of transience, 59.33%. Transience was found to be significant for the Virginia population, 54.41%, but not for the Kansas population, 51.00%. No difference in body weight was found between residents/transients for either population. Seasonal sex biases were found in both populations.

THE LIFE HISTORY FEATURES AND ECOLOGY OF *TENODERA SINENSIS* AND *T. ANGUSTIPENNIS* MANTISES IN EASTERN VIRGINIA. Cory A. Gall & Robert K. Rose, Department of Biology, Old Dominion University, Norfolk, VA 23529. Praying Mantises, *Tenodera sinensis* and *T. angustipennis*, are generalist predators located at the top of the arthropod food chain. These species inhabit eastern North America, and are common among old-field vegetation. Virginia is considered the southern limit of their natural range, with few field studies of these two mantises coexisting. My study was conducted on the Wildlife Refuge located on Virginia's Eastern Shore and is focused on their life history, movement, and species interaction in this region. Previous studies have shown that *T. sinensis* hatches earlier in the spring, giving it the ability to become larger in size than *T. angustipennis*. My initial results support these findings. In addition, I investigated the species ratio and sex ratio for both species throughout their lifecycle. I found that *T. sinensis* dominated for the majority of the year, but at the end of fall the species ratio was nearly 50:50. Past research has indicated *T. sinensis* and *T. angustipennis* hatch with 50:50 sex ratios; however, male presence are found to be greater until sexual maturity is reached, then females become more numerous. However, my results illustrate that *T. sinensis* and *T. angustipennis* sex ratios fluctuated throughout the life cycle, with *T. sinensis* and *T. angustipennis* females are dominating at different times in their growth season. My study also supports past research that females are more stationary to measured plots, while males often moved off measured plots.

A SMALL MAMMAL COMMUNITY IN A CHANGING LANDSCAPE IN SOUTHEASTERN VIRGINIA, 2005-2011. Jana Eggleston & Robert K. Rose. Department of Biological Sciences, Old Dominion University, Norfolk, VA 23508. In 2005 we began a monitoring program of the small mammal community on an old field site in southeastern Virginia. This site is a part of the Nature Conservancy Stewardship of lands adjacent to the Great Dismal Swamp National Wildlife Refuge, and a part of a greater wetland habitat restoration plan. An assortment of trees was planted on the site, drainage was altered, and vegetation succession was allowed to progress. We hypothesized this small mammal community would change from herbivorous old field species to those of forested wetlands, and that the numerically dominant species would change as succession progressed. Our study site was an 8 x 8 grid, with 12.5m intervals, and with two modified Fitch traps per station. We trapped for three days each month, averaging 4600 trap nights per year. Results to

date show the general decline of all old field species, the virtual disappearance of *Mus musculus* and *Oryzomys palustris*, stable densities of *Reithrodontomys humulus*, a shift in dominance from *Microtus pennsylvanicus* to *Sigmodon hispidus*, and most recently, the arrival of *Peromyscus leucopus*, a forest species.

THE EFFECTS OF VISUAL AND CHEMICAL CUES ON MALE MATE CHOICE IN EASTERN MOSQUITOFISH (*GAMBUSIA HOLBROOKI*). Nouman J. Rana & Lisa Horth, Dept. of Biol. Old Dominion Univ., Norfolk, VA 23529. Previous studies have illustrated that pheromones released by gravid females western mosquitofish (*Gambusia affinis*) increased male sexual activity during mating selection. However, little is known about the activities during mating selection in eastern mosquitofish (*Gambusia holbrooki*). In this study, we conducted experiments to determine (1) whether pheromones from gravid female mosquitofish induce different male activities as compared to non-gravid females and (2) if male mosquitofish prefer both a chemical and visual cues during mate selection. Our preliminary results indicate that pheromones released by gravid females are different from non-gravid females. In addition, gravid females induce a more active response from males during mate selection than non-gravid females. Also, males prefer to have both visual and chemical cues during the mate selection process. These results suggest that males are more likely to pick a female that they can visually see and one that has recently given birth as compared to one that cannot be seen and has not recently given birth.

EXPLORING THE ROLE OF THE C-TERMINAL REGION OF APQ12 IN THE REGULATION OF CELL CYCLE PROGRESSION AND CELL SEPARATION. James R. Oliver & Michael J. Wolyniak, Department of Biology, Hampden-Sydney College, Hampden-Sydney, VA 23943. The *Saccharomyces cerevisiae* peripheral nuclear membrane protein Apq12 is believed to play a critical role in the regulation of membrane fluidity and, ultimately, the way in which cells divide and separate. To further investigate the proposed role of Apq12 in these processes, we performed a series of site-directed mutagenesis experiments that attempted to delete the transmembrane domains of the protein as well as generate C-terminal truncations via nonsense point mutations. This allowed us to investigate if there was a specific domain of the Apq12 sequence that is necessary for its putative role in the regulation the cell cycle processes and cell separation. Our findings indicated that while Apq12 is, as a whole, a dispensable protein with respect to cell viability, the C-terminal region of the protein may provide a fine-tuned regulation of cell morphology, with different domains of this region acting in an antagonistic relationship to determine cell shape. Since we also found that cells deleted for Apq12 were slower in overall growth rates, our findings suggest that the C-terminal region of Apq12 may contribute to the efficiency of yeast cell cycle progression through the regulation of cell shape during the cell division process.

ISOLATION AND CHARACTERIZATION OF A NEW *CAENORHABDITIS* SPECIES TO BE USED IN EVOLUTIONARY AND DEVELOPMENTAL STUDIES. Borwyn A. Wang, Mejgan Mukhtarzada, Erin Haynes & Theresa M. Grana, Department of Biological Sciences, University of Mary Washington,

Fredericksburg VA 22401. The worm community of researchers is actively seeking a sister species for comparative genomics analysis for the identification of a *C. elegans* sister species. The purpose of this project was to isolate, identify, and characterize several new *Caenorhabditis* species that are phylogenetic relatives of the model organism *Caenorhabditis elegans*. Soil samples were collected in Fredericksburg, VA as well as in King George County in which nematodes were isolated and stocks were established on agar plates. Isolated nematodes were tested for model organism characteristics such as the ability to survive freezing, starvation, and male/female/hermaphrodite classifications. Nomarski microscopy was used to film mouth parts, pharyngeal bulbs, vulvas, and tails to further compare each species. Additional studies include filming embryo development and sequencing a part of the rDNA encoding the 18s ribosomal subunit to construct phylogenetic trees. This study was supported by UMW Undergraduate Research Grants and a VAS undergraduate grant.

EFFECT OF CpG OLIGONUCLEOTIDE AND LL-37 ON PROSTATE CANCER CELL GROWTH AND INVASION. April Lao<sup>1</sup>, Angela Gupta<sup>1</sup>, Maria Craig<sup>2</sup>, & Paul Deeble<sup>1</sup>, Departments of <sup>1</sup>Biology and <sup>2</sup>Chemistry and Physics, Mary Baldwin College, Staunton, VA 24401. CpG oligonucleotides, short pieces of DNA with characteristics of bacterial DNA, have recently been considered as an alternative to chemotherapy. Some studies found CpGs to stimulate anti-tumor immune responses with none of the side effects associated with traditional chemotherapy. However, CpGs effects are weak, and previous results are contradictory, with anti-tumor effects seen in ovarian cancer in mice but increased invasion and decreased growth in PC-3, LNCaP, and DU-145 prostate cancer cell lines. LL-37 is an antimicrobial DNA-binding peptide that increases the body's immune response to CpG. LL-37 is found throughout the body and in higher amounts in breast, lung, and prostate cancer tumors. The combination of CpG and LL-37 has recently been shown to enhance the anti-tumor effects seen with CpG in ovarian cancer in mice. In this project, the effect of CpG in combination with LL-37 on prostate cancer cell growth and invasion was investigated. The addition of LL-37 was hypothesized to enhance the effects seen with CpG (i.e. decreased growth and increased invasion). Experiments utilized androgen-independent PC-3 cells and androgen-responsive LNCaP cells. Results indicate the combination of CpG and LL-37 increases growth in PC-3 cells and increases invasion in LNCaP cells. These results could indicate that CpG oligonucleotides in combination with LL-37 should not be used as a therapy against prostate cancer.

NEUROENDOCRINE DIFFERENTIATION AND ITS EFFECTS ON CHEMORESISTANCE IN PROSTATE CANCER CELLS. Alex Kelly, Sophia Stone, Caitlin Combs, & Paul Deeble, Department of Biology, Mary Baldwin College, Staunton, VA, 24401. Neuroendocrine (NE) differentiation, induced by forskolin (Fsk) and 3-isobutyl-1-methylxanthine (IBMX), was analyzed in a prostate cancer progression model using androgen-responsive LNCaP and androgen-independent PC-3 prostate cancer cells. NE differentiation was quantified by measuring neuritic branch points and mean process length, and LNCaP cells were

found to exhibit a higher level of NE characteristics when compared to PC-3 cells. Cell viability in response to various chemotherapeutics, including Docetaxel and Rapamycin, was measured in undifferentiated and NE differentiated cancer cell lines using an MTT assay. NE differentiation was found to protect LNCaP cells from cell death induced by chemotherapeutic agents.

EFFECTS OF PHENOLIC ACIDS ON OVIPOSITIONAL SELECTION IN THE CABBAGE WHITE BUTTERFLY, *PIERIS RAPAE*. Jessica L. Bray, Mary E. Lehman & Amanda J. Lentz-Ronning, Department of Biological and Environmental Sciences, Longwood University, Farmville VA 23909. *Pieris rapae* oviposit on plants that are most suitable for larval growth and survival. The presence of a variety of plant secondary metabolites may be one factor in determining ovipositional choices. Phenolic acids are secondary metabolites that are widespread among plants, but their effects on *Pieris rapae* have not been well characterized. Wisconsin Fast Plants were treated with four different phenolic acids (1.0 mM concentrations) to see if they would deter or stimulate oviposition. Female butterflies were provided a choice of whether to lay eggs on the control plants sprayed with deionized water or the plants sprayed with a phenolic acid. *p*-coumaric acid significantly stimulated oviposition, whereas salicylic acid and protocatechuic acid had no significant effect. With the removal of one extreme outlier from the data set, the stimulatory effect of ferulic acid was also significant. Ferulic acid and *p*-coumaric acid are very similar in chemical structure and preliminary experiments suggest that *p*-coumaric acid increases larval growth.

EFFECTS OF HOST PLANT ALLELOCHEMICAL AND NUTRIENT STATUS ON THE CABBAGE WHITE BUTTERFLY, *PIERIS RAPAE*. Kristen S. Walker, Mary E. Lehman & Amanda J. Lentz-Ronning, Department of Biological and Environmental Sciences, Longwood University, Farmville VA 23909. *Pieris rapae* is known to detect the chemical content of plants that would be best suited for offspring development. Plant allelochemical and nutritional status were manipulated to determine the main and interactive effects on ovipositional choices of *Pieris rapae*. Wisconsin Fast Plants were supplied with Hoagland's nutrient solution at concentrations of 1/2X (control) or 1/8X (reduced nutrient treatment). Plants were sprayed with deionized water (control) or 1.0 mM *p*-coumaric acid (PCO; allelochemical treatment). The effects of PCO and nutrient concentration were first assessed individually and then in an interaction experiment. Females and plants were placed in light boxes and eggs were counted after a 24- hour period. A significantly higher number of eggs were deposited on the 1/2X control plants (mean  $\pm$  SE:  $21.7 \pm 5.5$ ) compared to the 1/8X reduced nutrient treatment ( $9.5 \pm 2.8$ ). Females oviposited significantly more eggs on the PCO-treated plants ( $35.3 \pm 3.7$ ) than on the DI water controls ( $25.2 \pm 4.4$ ). In the interaction experiment, only the main effect of nutrient concentration was significant. PCO's lack of significance and the lack of a significant interaction may be due to the low number of replicates (N=6). Overall, results suggest that female *Pieris rapae* can distinguish the nutrient status of plants and that this is a stronger determinate of ovipositional choice than host plant allelochemicals.



THE ROLE OF INTERFERON GAMMA IN EXPRESSION OF KLF4 IN LUNG FIBROBLASTS. H. Rushdi<sup>1</sup>, E. Quraishi<sup>1</sup>, S Qureshi<sup>1</sup>, J. Forrest<sup>1</sup>, S.D. Nathan & G Grant<sup>1</sup>, <sup>1</sup>SSB GMU, Manassas VA, <sup>2</sup>IHVI Inova Fairfax, Falls Church, VA. Krüppel like 4 Factor (KLF4) is a member of the zinc finger transcription factor family which is involved in cells' transcriptional response to multiple critical stimuli, such as DNA damage, growth stimulation and differentiation. KLF4 activity is cell-type dependent acting as both an inducer and repressor of gene expression. It is predominantly associated with post-mitotic terminally differentiated/ing cells, playing a major role in cell homeostasis by induction of p21 and inhibition of p53. However KLF4 can also act as a tumor promoter in cells that have bypassed the p21 checkpoint. Idiopathic pulmonary fibrosis (IPF) is a terminal lung disease propagated by the fibroblasts. IPF fibroblasts over-express KLF4 *in vivo*. Therefore we investigated the potential role of KLF4 overexpression in IPF by inducing its expression with Interferon gamma (INF $\gamma$ ). IPF and normal fibroblasts were seeded at  $2.5 \times 10^5$  cells per 60 mm dish in 10% DMEM at 37°C in a 5% CO<sub>2</sub> incubator. Cells were serum starved for 16 hours to synchronize prior to addition of INF $\gamma$  at 0, 200 and 400 Units per ml for 24 hours. RNA was extracted (RNeasy Kit Qiagen) and cDNA generated (iscript BioRad) from 1 $\mu$ g of total RNA. Real Time Quantitative PCR was carried out for KLF4 induction using ribosomal 18S as a normalization control. INF $\gamma$  exposure of IPF and normal fibroblasts resulted in increased expression of KLF4 protein which may influence the proliferative and survival behavior of these fibroblasts in the IPF lung.

INVESTIGATIONS OF THE EFFECT OF THYMOSIN-BETA4 ON LUNG FIBROBLAST SURVIVAL. S. Qureshi<sup>1</sup>, J. Forrest<sup>1</sup>, H. Rushdi<sup>1</sup>, E. Quraishi<sup>1</sup>, S.D. Nathan<sup>2</sup> & G Grant<sup>1</sup>, <sup>1</sup>SSB, GMU, Manassas VA, <sup>2</sup>IHVI Inova Fairfax, Falls Church VA. Idiopathic Pulmonary Fibrosis (IPF) is a fatal lung disease, with no therapy and no cure. IPF is propagated by over abundant pulmonary fibroblasts which deposit excessive extracellular matrix (ECM) causing damage to the alveoli. The excessive ECM interferes with gaseous exchange and ultimately results in organ failure. Thymosin Beta 4 (T $\beta$ 4) is a tiny cellular protein with hormone like properties. Initially identified as a cytoskeletal protein, it has been recently identified with wound healing, cell migration, angiogenesis, and anti-apoptosis. T $\beta$ 4 is upregulated in IPF cells compared to normal lung fibroblasts. In this experiment we investigated the effect of T $\beta$ 4 to prevent hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induced apoptosis in primary IPF and normal fibroblasts. IPF and normal fibroblasts were exposed to lethal doses of H<sub>2</sub>O<sub>2</sub> in the presence and absence of T $\beta$ 4 for 24 hours in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum in a 96 well format. After 24 hours of exposure cell survival was determined by the Acid Phosphatase and MTT cell proliferation assay. T $\beta$ 4 exposure increased cell survival in both normal and IPF cells. Results indicate that T $\beta$ 4 has the potential to play a significant role in preventing apoptosis in IPF lung fibroblasts. This cytoprotective property of T $\beta$ 4 may contribute to the overall abundance of fibroblasts in the IPF lung, a hallmark and contributing factor of this disease. Further investigations into the action of this protein may lead to therapeutic and biomarker discoveries which may aid in preventing and treating IPF.

THE *DROSOPHILA* SPECTRAPLAKIN SHORT STOP DIFFERENTIALLY REGULATES CLASS SPECIFIC DENDRITE MORPHOGENESIS. James Boddu, Sarah A. Trunnell, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Disorders of the nervous system can often be attributed to developmental abnormalities occurring during neurogenesis, which affect the morphology, and ultimately functionality of neurons. The *Drosophila* peripheral nervous system (PNS) provides an excellent model system in which to elucidate the molecular mechanisms governing dendrite morphogenesis. Spectraplakins are an evolutionarily conserved family of cytoskeletal cross-linking proteins that link the actin and microtubule cytoskeletons. Cytoskeletal structure and organization are key mediators of neuronal shape, and by extension neuronal function. To address the role of Spectraplakins in dendrite morphogenesis, we focused on the sole known Spectraplakins gene in *Drosophila* referred to as *short stop* (*shot*). Here we demonstrate that *shot* is present in all classes of dendritic arborization (da) neurons where it exerts differential effects on class specific dendrite morphogenesis. Comparative morphological analyses reveal *shot* is required to restrict dendritic complexity among the simpler class I and II da neurons, whereas *shot* is required to promote dendritic complexity among the more complex class III and IV da neurons. These results suggest dendrite morphogenesis is subject to context-dependent regulation mediated via *shot*.

INVESTIGATING THE ROLE OF RNAi REGULATION IN CLASS-SPECIFIC DENDRITE MORPHOGENESIS. Myurajan Rubaharan, Eswar P.R. Iyer, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Dendrites function as the primary sites of synaptic and/or sensory input and integration in the developing nervous system. The initiation and maintenance of dendritic arbors determine both the number and type of inputs they receive and thus is a critical determinant in establishing functional neural networks. Despite their functional importance, the molecular mechanisms governing cell-type specific dendrite morphogenesis remain largely unknown. One emerging mechanism involves the regulation of differential gene expression in neuronal subclasses via the evolutionarily highly conserved RNA interference (RNAi) pathway. The *Drosophila melanogaster*, peripheral nervous system, an excellent genetic and morphological model system for studying class specific dendrite morphogenesis, was used to perform loss-of-function (LOF) and gain-of-function (GOF) phenotypic studies to understand the role(s) of ten key genes essential for mediating RNAi in the fruitfly. These studies provide novel mechanistic insight into the role RNAi pathway plays in controlling class-specific da neuron dendrite morphogenesis. The broader implications of these studies are related to the relative contribution of this conserved pathway in controlling gene expression at the class-specific neuronal level in mammalian systems and how this regulation ultimately contributes to acquisition of distinct neuronal morphologies that underlie the establishment of complex neural networks.

FORAGING AT THE SNAIL DINER: THE EFFECTS OF PARASITE INFECTION ON SNAIL FORAGING BEHAVIOR. Sabrina Brooks<sup>1</sup>, Jeremy M. Wojdak<sup>2</sup> & Lisa K. Belden<sup>1</sup>, <sup>1</sup>Virginia Tech, Department of Biological Sciences, Blacksburg, VA 24060, <sup>2</sup>Radford University, Department of Biology, Radford, VA 24142. Snails are common hosts of parasitic flatworms (trematodes). Snails typically feed at dawn and dusk, and during this time they are especially vulnerable to predators. Parasite infections can alter this feeding pattern, which can increase the probability of infection or transfer of the parasite to the next host. We examined how infection by *Echinostoma trivolvis* can affect the foraging behavior of *Helisoma trivolvis* snails. In separate trials, we examined foraging behavior in (1) snails infected as first intermediate hosts and (2) snails either exposed or not exposed to cercariae (second intermediate host infection). We used two assays to assess foraging behavior. First, we examined how the frequency of snails on the tiles changed over twelve hours and the rate at which the snails in the different infection groups reached the tiles. The second assay compared the amount of lettuce consumed within 25 hours among snails in the different infection groups. We did not see a difference in proportion of snails on the resource tiles between cercariae exposed and non-exposed snails. However, for the first intermediate host snails, the proportion on the tiles was higher after 120 minutes and remained higher until the end of the trial. For the lettuce trials, there was no difference in the amount of lettuce consumed among the three categories of snails. While there were some differences in foraging behavior between first and second intermediate host snails in our first assay, we did not see dramatic difference in foraging based on infection status in *Helisoma* snails.

SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA* BIOFILM TO ALPHA-HELICAL PEPTIDES: D-ENANTIOMER OF LL-37. Scott N. Dean, Barney M. Bishop & Monique L. van Hoek, Department of Systems Biology, George Mason University, 10900 University Blvd., Manassas, VA 20110. *Pseudomonas aeruginosa* is a highly versatile opportunistic pathogen and its ability to produce biofilms is a direct impediment to the healing of wounds and recovery from infection. Interest in anti-microbial peptides has grown due to their potential therapeutic applications and their possible use against antibiotic resistant bacteria. We tested the human AMP, LL-37, and the effect of a protease-resistant LL-37 peptide mimetic, the peptide enantiomer D-LL-37, for anti-microbial and anti-biofilm activity against *P. aeruginosa*. The CD spectra of D- and L-LL-37, and the trypsin resistance of D-LL-37 was confirmed. The helical cathelicidin from the cobra *Naja atra* (NA-CATH), and synthetic peptide variations (ATRA-1, ATRA-2, NA-CATH:ATRA1-ATRA1) were also tested. Although the cobra cathelicidin and related peptides had strong anti-microbial activity, they did not inhibit *Pseudomonas* biofilm formation. D-LL-37 inhibited attachment, promoted *Pseudomonas* motility, and decreased biofilm formation by altering the rate of twitching and downregulating the expression of the biofilm related genes, *rhlA* and *rhlB*. D-LL-37 protected *Galleria mellonella* *in vivo* against *Pseudomonas* infection, while NA-CATH:ATRA1-ATRA1 peptide did not. This study shows D-LL-37 is able to promote bacterial twitching motility and inhibit biofilm formation, and protect

against infection, and suggest that this peptide may be a critical component for the development of new treatments for *P. aeruginosa* infection.

**CHARACTERIZATION OF DOWNSTREAM EFFECTORS MEDIATING CUT TRANSCRIPTIONAL REGULATION OF CLASS-SPECIFIC DENDRITE MORPHOGENESIS.** Luis Sullivan, Eswar P.R. Iyer, Madhu Karamsetty, & Daniel N. Cox, School of System Biology, Krasnow Institute for Advanced Study, George Mason University, Fairfax VA 22030. Neuronal form dictates function and in a circuitry as complex as the human brain the post-synaptic properties of the neuron are established in large part by dendritic morphology. Transcriptional regulation has emerged as a pivotal mediator of class specific dendrite morphogenesis; however, the downstream effectors of these transcription factors remain largely unknown as are the cellular events that direct morphological change. Recent studies have implicated the *Drosophila* homeodomain transcription factor Cut and its vertebrate homolog in mediating dendrite morphogenesis in the peripheral and central nervous systems. To characterize putative transcriptional targets of Cut regulation, a genetic suppressor screen has been performed in which Cut overexpression has been coupled with target gene-specific *in vivo* RNAi knockdown. Preliminary analyses have identified >400 genes that represent potential direct targets of Cut regulation in *Drosophila* dendritic arborization (da) neurons. Here we report the discovery of target genes that either suppress or enhance Cut-mediated effects on da neuron dendritic morphology. The molecules uncovered in our screen cover a broad range of biological functions. Collectively, these analyses reveal novel transcriptionally regulated pathways and cell biological processes essential to the specification of class specific dendritic morphologies.

**THE DETECTION OF MYCOPLASMA SPECIES CONTAMINATION IN CULTURED HUMAN CELLS.** Amy Yu, Pranvera Ikononi, Nadine Kabbani, Ancha Baranova & Aybike Birerdinc, College of Science, George Mason University, Fairfax, VA. *Mycoplasma* can contaminate eukaryotic cell cultures. It can alter reproducibility of experiments or completely destroy the cell line. In most of the cell culture labs, only one or a few species of *Mycoplasma* are systematically monitored. The novel Universal Mycoplasma Detection Kit from American Type Culture Collection (ATCC) provides a way to detect over 60 species of *Mycoplasma*. **AIM:** To perform field test of the Universal Mycoplasma Detection kit using continuous cell cultures. **Methods:** The kit contains reagent for a PCR reaction using universal primers that are specific to the 16S rRNA coding region in the mycoplasma genome, allowing to specifically amplify *Mycoplasma* DNA. If a cell culture is contaminated, after gel electrophoresis, the presence of a 464-bp amplicon is detected. In our experiment, 8 PCR reactions were performed for each cell culture, including sample A, B and C provided by the kit as reference examples of different levels of mycoplasma contamination; two test samples; positive control; purified *Mycoplasma* DNA, positive control with lysate to confirm that inhibition of PCR by the cell lysate did not occur; and negative control. **Results:** Cell culture samples from Dr. Kabbani's and Dr. Van Hoek's lab were negative for *Mycoplasma*. Sample B, Sample C, positive control and positive control with lysate all showed a band at 464-bp. Sample A, all test samples and negative control showed no bands. This confirms the absence

of *Mycoplasma* in the cell cultures and proves that ATCC kit is reliable, easy to use and can be recommended for introduction to the market.

ADAR FACILITATED RNA EDITING IN HUMAN PLASMACYTOID DENDRITIC CELLS (PDC). A. Sharma<sup>1</sup>, K. Doyle<sup>1</sup>, M. Connors<sup>4</sup>, A. Patamawenu<sup>4</sup>, P. Gillevet<sup>3</sup>, A. Biredinc<sup>1,2</sup>, & A. Baranova<sup>1,2</sup>, <sup>1</sup> School of Systems Biology, George Mason University, Fairfax VA 22030, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA 22042, <sup>3</sup> Microbiome Analysis Center, George Mason University, Manassas, VA 20100, <sup>4</sup>National Institute of Health, Bethesda, MD 20892. Adenosine (A) to Inosine (I) RNA editing is facilitated by enzymes known as ADAR (Adenosine Deaminase that Act on RNA). ADARs specifically recognize double stranded RNA structures or RNA duplex structures as their substrates. Inosine is translated as Guanosine, since most enzymes recognize Inosine as Guanosine. Examples of physiological ADAR editing are edits to neuronal Glutamate and Serotonin receptor transcripts. Here we set to find out whether ADAR-editing in human PDCs (Plasmacytoid Dendritic Cell) is limited to TLR7, or whether it covers other known ADAR targets, including other TLR receptors, FLNA, IGFBP7, KCNA1, GABRA3, and CYFIP2. Site specific primers around previously known edited sites were designed using NCBI primer blast and then tested on cDNA derived from universal RNA and adipose tissue. cDNAs from purified PDC cells will be used as templates for PCR amplification, tagged, purified and subjected to Multitagged (MTPS) pyrosequencing on Roche GS-FLX instrument. If successful, these experiments will be the very first demonstration that RNA editing activity is present in PDCs and acts on physiologically important gene targets.

USP22 ACTIVITY REQUIRED FOR EFFICIENT mRNA 3' PROCESSING OF STAT1 REGULATED GENES. Edmond Chipumuro and Melissa A Henriksen Department of Biology, University of Virginia, Charlottesville VA 22904. Histone modifications represent a major mechanism of eukaryotic transcriptional regulation at multiple levels. There is increasing experimental evidence that histone H2B monoubiquitination (ubH2B) and its deubiquitination are both involved in gene activation. Recent findings have suggested that, the ubiquitin specific peptidase 22 (USP22) regulates c-MYC and p21 expression and is required for appropriate cell cycle progression. However, a mechanistic understanding of USP22 function in gene expression remains elusive. Here, we utilized the rapid and transient STAT1 activation of the *IRF1* gene to characterize roles of USP22. RNAi mediated depletion of USP22 significantly increased *IRF1*-associated levels of ubH2B and down regulates *IRF1* expression. Importantly, USP22 knockdown consistently resulted in inefficient mRNA 3' processing of the *IRF1* gene and led to increased read through RNA polymerase II. Furthermore, depletion of USP22 resulted in lower occupancy of RNA Pol II phosphorylated at serine 2 (CTDS2) and cleavage and polyadenylation specificity factor 73 (CPSF 73) on the *IRF1* gene body and it's read through. Taken together, our results suggest that, USP22 activity is required during *IRF1* transcription to regulate co-transcriptional processing of the mRNA.

DEVELOPMENT OF A SIMPLIFIED METHOD FOR DETECTING NUCLEAR IMPORT. Adil Quraish & Stephen Gallik, Ph. D., University of Mary Washington. Nuclear import is an important cellular process through which proteins enter the nucleus through the nuclear pore complex. It is commonly detected and studied using sophisticated protocols too time-consuming, complicated and expensive to be routinely used in the undergraduate teaching or undergraduate research laboratory. The specific objective of the study reported here is to develop a simplified method for the detection of nuclear import that can be more easily used in an undergraduate setting. The simplified approach taken here involves homogenization of rat liver, incubation of the homogenates with commercially-available fluorescently-labeled proteins, isolation of the rat liver nuclei from the homogenates using a standard Optiprep density gradient centrifugation techniques, followed by the fluorescent-microscopic inspection of the isolated nuclei. Results showed nuclei isolated from homogenates incubated with fluorescently-labeled proteins conjugated to a 13-amino acid nuclear localization signal (NLS) fluoresced, showing nuclear import of the fluorescent protein, while nuclei isolated from homogenates incubated with fluorescently-labeled proteins lacking an NLS did not fluoresce.

THE EFFECT OF VEGF ON ADIPOSE STEM CELL MIGRATION. Laila Almahdali & Kathryn E. Loesser-Casey, Dept. of Biol., University of Mary Washington, Fredericksburg VA 22407. Stem cell research is one of the cutting edge ways scientists and clinicians hope to treat human diseases in the future, but stem cells are difficult to obtain in sufficient quantities. In order for stem cells to be of any use following a myocardial infarct or similar injury, they must be able to migrate to the place of damage. It has been found that growth factors such as Vascular Endothelial Growth Factor (VEGF) can assist in the migration pathway by stimulating endothelial growth, migration and blood vessel formation. It is also found the VEGF is secreted by adipose stem cells, leading to the possibility that VEGF may be necessary for stem cells release or migration. The goal of this experiment was to determine if treatment with 50ng/ml VEGF increased migration and whether the age of the stem cells affected migration. Cells were collected from both young (10 to 12 week) and old (over 30 week) CF-1 mice and used for migration assays using Boyden blind-well chambers. The number of cells that had migrated towards media with or without VEGF was counted and data evaluated using a one-sample T-test. Cells isolated from the younger mice appeared to show an increase in cell migration towards the VEGF-containing media; however statistics indicates no significant change in cell number over the control. When cells from adult mice were tested we saw a decrease in cell migration, but no statistics can be performed on this data because only one assay was performed due to very slow cell growth. We plan to repeat these experiments, changing the cell number used for each migration and increasing the concentration of VEGF to get more consistent results.

**Biomedical and General Engineering**

DIFFERENTIATING MANIFESTATIONS OF SOPITE SYNDROME AS PRODUCED BY INERTIAL MOTION AND VECTION. Brittany N. Neilson & J. Christopher Brill, Department of Psychology, Old Dominion University, Norfolk VA 23529. Sopite syndrome is a polysymptomatic manifestation of motion sickness that presents itself as marked drowsiness despite receiving an adequate night's sleep, relaxation, and lack of motivation. Previous studies evaluate sopite syndrome using either a motion device or a vection device. The present research aims to differentiate the symptoms of sopite syndrome produced by actual motion versus perceived self-motion (i.e., vection) in order to better identify the psychophysiological markers of sopite syndrome. Twenty-five undergraduate psychology students participated in the pilot study. An analysis of covariance was used to evaluate participant's self-reports on motion questionnaires, physiological measurements, and brain activity across three motion conditions (inertial motion, vection, and control) holding traits of sleepiness and motion sickness constant. Participants in the motion condition reported increased symptoms ( $M = 20.75$ ,  $SD = 23.95$ ) compared to participants in the vection ( $M = 8.53$ ,  $SD = 25.45$ ) and control conditions ( $M = 5.53$ ,  $SE = 24.25$ ), but this effect was not statistically significant at  $p < .05$ ,  $F(2, 19) = 2.87$ ,  $p = .081$ ,  $\eta^2 = .20$ , due to insufficient sample size. Implications for future research will be discussed.

SEGMENTATION OF INTERFACE PRESSURE MAPS: THE USE OF A CONNECTED ELLIPSE MODEL TO IDENTIFY AREAS AT RISK FOR PRESSURE ULCER DEVELOPMENT. Anatheia A. Pepperl and Paul A. Wetzel, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284. Mary Jo Grap, Department of Adult Health and Nursing Systems, Virginia Commonwealth University, Richmond, VA 23298. In order to study pressure ulcer development, it is useful to analyze pressures at specific high-risk areas, or regions of interest (ROIs). A contour-based connected ellipse model was used to segment ROIs. A user selected the approximate centers of ROIs. An Expectation-Maximization algorithm refined the center locations and defined an ellipse at each ROI. The connected ellipse model was then iteratively refined so as to minimize (1) the distance between connected ellipses and (2) the distance between the image contour and the edge of the closest ellipse. Ten pressure images were collected from four patients in intensive care units at the Virginia Commonwealth University Health System (VCUHS). The distance between ROI peak pressures generated by automatic and manual segmentations was defined as the error. The average errors (in inches) for each ROI were: 6.64, left scapula; 7.76, right scapula; 4.54, left trochanter; 5.31, right trochanter; 0, left heel; 0.85, right heel. The average error was 4.18 inches. Visual inspection showed that this error was caused by the peak pressure lying just beyond the contour of the ellipse. The connected ellipse model is an effective method for segmenting pressure images. The model may be further refined through the inclusion of intensity data.

DEVELOPMENT OF A NOVEL BLOOD ANALOG WITH PHYSIOLOGICAL SHEAR-THINNING AND VISCOSITY CHARACTERISTICS. S. J. Warren, C. E. Taylor, and G. E. Miller, Dept. Biomedical Engineering, VCU. Mock loops are an integral part of the testing and evaluation of cardiac assist device. In order to obtain near physiological data in a mock circulatory loop, a compressive fluid similar to human blood is needed. This project will test the properties of an oil base fluid, in order to match the viscoelastic properties of Human blood. The work presented was based on the following research questions: Does the oil based fluid show shear thinning properties closest to those of human whole blood? Does the oil based fluid correlate with the viscoelastic curve of human whole blood better than other blood analogs currently in use? All viscosity data was collected on an Anton Paar Rheometer with a parallel plate and 500 micron gap. The test was a shear sweep from .001 to 1000 1/s. The work presented two candidates; one that is optical clear and the other is rheological more accurate. The components are pure and commercially validated for mechanical properties, which releases the end user from having to revalidate the constructed solution. Choice of fluid construction was designed to prevent large dilution factors, to provide a robust method for fabricated a shear-thinning blood analog for cardiac assist device evaluation.

CONTROL MODEL AND PERFORMANCE OF A COMPLIANCE CHAMBER FOR SIMULATING ARTERIAL SYSTEMIC COMPLIANCE. C. E. Taylor and G. E. Miller, Dept. of Biomedical Engineering, VCU. Utilizing mock circulatory loops as a bench test method for cardiac assist technologies provides that the system must be capable of reproducing the circulatory conditions that would exist physiologically. Of particular interest is the ability to determine compliance delivered by the system, and the ability to change the compliance in real-time. The latter capability allows for continuous battery testing of conditions without stopping the loop operation when changing the compliance chamber settings. The compliance chamber presented utilizes a natural latex rubber membrane separating the fluid and air portions of the device. Change in system compliance is affected by the airspace pressure, which creates more reaction force at the membrane to the fluid pressure. A pressure sensor in the fluid portion of the chamber and a displacement sensor monitoring membrane position allow for real-time inputs to the control algorithm. A predefined numerical model correlates the displacement sensor data to the volume distention of the membrane. The PI controller tuning was achieved by creating a computational model of the compliance chamber using Simulink Simscape toolboxes. It was found that the resulting control architecture was capable of maintaining a compliance set point, and allowed for changes to the compliance without stopping the pulsatile flow of the mock circulatory loop.

THE EVOLUTION OF AN EVENT DETECTION ALGORITHM. Pallavi Ramnarain and Dr. Paul A. Wetzel, Dept. of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA. The purpose of this presentation was to explore the methodological processes behind the development of event detection algorithms. Signal acquisition imposes the first set of limitations on event detection. After that an iterative process is initiated. The first step is to identify the event of interest. Then it must be defined using finite, quantitative characteristics that



distinguish it from the remainder of the signal. Then an approach to detection can be developed. The approach must be validated and then separately evaluated for its accuracy. Limitations are identified and quantitatively defined, and so the process continues. To illustrate this example data from a study examining preterm infant bottle feeding competency was used. The focus was placed on the detection of bottle sucking activity. Multiple approaches to event detection were explored.

A PATIENT SPECIFIC COMPUTATIONAL MODEL OF ADULT ACQUIRED FLATFOOT DEFORMITY. E.M. Spratley & J.S. Wayne, Dept. of Biomedical Engineering & Orthopaedic Surgery, VCU, Richmond, VA 23284. Computational modeling offers advantages over MRI and plane-film x-ray alone in the quantification of adult acquired flat foot deformity, (AAFD). As such, a population of solid body models was created for the purpose of replicating common radiographic tests used in the quantification of degenerating flat foot morphology. This was accomplished by acquiring a sample of sub-millimeter resolution MRI scans from a group of clinically diagnosed Stage II AAFD sufferers prior to surgery. The scans were imported into the medical imaging package, Mimics (Materialise, Ann Arbor MI), wherein the bony surfaces were isolated from the surrounding tissues and triangulated to form a solid bodies for each bone in the foot. Subsequently, the bodies were imported into SolidWorks (SolidWorks Corp., Concord MA) and templates recreating an oblique anteroposterior film x-ray view were constructed. This template allowed the talo-1<sup>st</sup> metatarsal angle (T1MT) and talo-navicular coverage angle (TN) to be calculated for each of the AAFD models as well as a non-flat foot cadaver-based model that served as the control. The normal model had a T1MT=5.35° and a TN=0.76°. The AAFD models had a mean T1MT=8.28 ±1.66° and a TN=5.15±4.65°. While these values are lower than those in the literature for T1MT and TN, the values for T1MT were within ranges reported clinically. Additionally, the sample set was able to capture the characteristic increase in the two angles for AAFD feet over those of non-afflicted feet. Further refinement of these models could lead to better classification of AAFD patients with the ultimate goal of improving the treatments prescribed.

## Botany

GROWTH AND NUTRIENT ACCUMULATION RESPONSES TO PHOSPHORUS DEFICIENCY IN CELLULOSE SYNTHASE MUTANTS OF *Arabidopsis thaliana*. Rob Harbert, Roanoke College. Cellulose synthase mutants of *Arabidopsis thaliana* are affected not only in the overall amount of cellulose they are able to produce, but also in the carbohydrate composition of their cell walls. These alterations in the composition of the cell wall may also impact the functions of other components of the cell wall and plasma membrane. The cellulose synthesis mutants JE100, *rswl-1*, and *prcl*, as well as the Columbia and Wassilewskija (WS) wild types, have been used to see if changes in cell wall composition have any effect on how these mutants perform in phosphorus deficient conditions. The results indicate that JE100 plants respond differently to

phosphorus than the wild types or possibly the other mutants. The results suggests further, that cell wall alterations may have an effect on how nutrients enter roots, or how growth responses are processed. Ward et. Al. (2008) suggested that the phosphorus deficiency dependent growth responses occurred as a result of iron over-accumulation through an interaction that is not well understood at this time. This observation was used to test whether or not the cell wall alterations in the JE100 plants affect how nutrients get across the cell wall and into the root. To do this, phosphorus and iron content was measured in dried plant tissues for Columbia and JE100 plants grown on the four phosphorus conditions on ATS media. It was observed that while the JE100 plants follow the same iron accumulation trend as Columbia, the JE100 plants accumulated more of both nutrients in all scenarios than the Columbia plants do. While this observation does not explain the JE100 plant's relative insensitivity to phosphorus deficiency, it does imply that the JE100 mutation alters the cell wall in such a way that nutrient uptake and accumulation appear to be affected. It is, however, unclear what the nature of this interaction is, or if this affects how nutrient uptake across the cell wall (the apoplast) is affected.

STATUS AND TRENDS OF PHYTOPLANKTON POPULATIONS IN VIRGINIA WATERS AND CHESAPEAKE BAY. H.G. Marshall, T.A. Egerton, M. Semcheski, & M. Lane. Dept. Biological Sciences, Old Dominion University, Norfolk, VA 23529-0266. Results of monitoring phytoplankton populations the past 25 years was presented with emphasis placed on bloom producing species in Virginia tidal tributaries. Many of these blooms have been associated with reduced oxygen levels in these waters and fish kill events. Potentially harmful taxa, including known toxin producers, of major concern in these waters include *Cochlodinium polykridoides*, *Alexandrium monilatum*, *Karlodinium veneficum*, *Microcystis aeruginosa*, *Chattonella subsalsa*, and *Prorocentrum minimum*. *C. polykrikoides* and *K. veneficum* blooms were becoming more common, with *A. monilatum* and *C. subsalsa* blooms of more recent development regarding their regional impact. *P. minimum* has shown a significant long-term trend of increased abundance over the last 2 decades within the lower Chesapeake Bay. Algal blooms by a variety of other taxa also occurred seasonally. A survey of 19 Virginia lakes in 2010 also indicated presence of several potential toxin and bloom producing species at 8 of these lakes. These were *Anabaena spiroides*, *Aphanizomenon flos-aquae*, *Cylindrospermopsis raciborskii*, *Limnithrix redekei*, *Microcystis aeruginosa*, and *M. wesenbergii*. In summary, algal blooms are common in Virginia waters and Chesapeake Bay. They are produced by a diverse group of algae, some of which are toxin producers. There is evidence that several of these algae have expanded their range and duration of bloom formation; including the presence of bloom producing invasive species entering Virginia waters and the Chesapeake Bay. Funding support for this study came from VDEQ and VDH.

COMMUNITY-MEDIATED CONSERVATION IN THE SOUTH PACIFIC: HOW HUMAN INFLUENCE OVER THE PAST MILLENNIA HAS SAVED A RARE TREE SPECIES FROM POSSIBLE EXTINCTION. Helen K. McKinney<sup>1</sup>, Ryan D. Huish<sup>1</sup> & Joseya Mateboto<sup>2</sup>, <sup>1</sup>Department of Biology, Hollins University, Roanoke, Va, <sup>2</sup>Fiji Forestry Department, Kadavu Island, Fiji. Community-mediated

management strategies for indigenous resources, employed by various communities, have shown success on local levels despite overall trends of resource decline. While the history of sandalwood trade in the South Pacific is rife with over exploitation, ancient cultural practices and modern management techniques for native *Santalum yasi* by Fijian and Tongan communities may have actually enabled *S. yasi*'s survival despite the continuing threat of over harvesting and habitat destruction. Remaining wild *S. yasi* stands lack genetic insularity typical of island populations, suggesting significant migratory events, such as the pre-historic movement of seeds and seedlings across and between Tonga and Fiji via trade, and marriage practices as documented by early explorers to these regions. Furthermore, current strategies employed by a Fijian village in the management of *S. yasi* reveal foundational principles that can guide the development of effective management of other endangered economic resources and curtail common problems of implementation. The inclusion of all community members, in the benefits and responsibilities of resource tenure and stewardship, enables better prevention of poaching, and fiscal community interests. Further research and application of these, and similar practices in other areas, may help resolve current management challenges to the preservation of culturally and economically valuable species around the world.

ANTIMICROBIAL PROPERTIES OF THE TONGAN MEDICINAL PLANT *SYZGIUMCORYNOCARPUM*: BIOASSAYS IN SUPPORT OF ETHNOBOTANICALLY-GUIDED RESEARCH. Erika Schooley & R. D. Huish, Dept of Biology, HU, Much of the traditional medicine of Tonga specifically treats infections. In a previous study, *Syzygium corynocarpum* not only showed significant inhibition rates of *Staphylococcus aureus* in laboratory bioassays, but was also the plant most frequently referenced by Tongan healers for use in treating infections. This correlation alludes to the benefits of ethnobotanically-guided drug research. Furthermore, in medicinal preparations, Tongan healers specifically use the young leaves of *S. corynocarpum*, which are a characteristic red-purple color changing to dark green at maturity. Extracts of mature leaves and bark, as well as the young leaves, are being tested for inhibition rates of *S. aureus* to compare efficacy and strengthen the case for ethnobotanically-guided research in the search for novel drugs.

CURRENT POPULATION DYNAMICS OF A RARE SANDALWOOD TREE (*SANTALUM YASI*) IN FIJI AND TONGA. Ryan D. Huish<sup>1</sup>, L. Thomson<sup>2</sup>, T. Faka'osi<sup>3</sup>, H. Likiafu<sup>3</sup>, M. Tuiwawa<sup>4</sup>, J. Mateboto<sup>5</sup>, & D. Little<sup>6</sup>, <sup>1</sup>HU, <sup>2</sup>Secretariat of the Pacific Community, <sup>3</sup>Tongan Ministry of Forests, <sup>4</sup>USP, <sup>5</sup>Fiji Dept. of Forestry, <sup>6</sup>NYBG. Valued internationally for the aromatic oil found within its heartwood, Sandalwood (*Santalum*, Santalaceae) is one of the most heavily exploited groups of plants across its range—Oceania, Southeast Asia, and India. One of the most valued sandalwood species is *Santalum yasi*, harvested extensively in Fiji and Tonga. To help aid in conservation efforts for this now rare tree, population dynamics, current species distribution, and ecological threats were investigated. It was concluded that the few remaining wild stands display discontinuous size class structures, are under regenerative stress, and that the natural distribution has diminished significantly, even to local extinction in some areas. Several threats appear to be affecting the

population dynamics of *S. yasi*, including destructive harvesting and invasive species. It is suggested that Forestry and governmental efforts focus on the promotion of local involvement in assisted natural regeneration of wild stands through community-mediated conservation.

A METHOD FOR LONGLEAF PINE TIMBER IDENTIFICATION AND ITS APPLICATION TO RELICTS IN SOUTHEASTERN VIRGINIA. Thomas Eberhardt<sup>1</sup>, Philip Sheridan<sup>2</sup>, Arvind Bhuta<sup>3</sup>, <sup>1</sup>USDA Forest Service, Pineville, LA, <sup>2</sup>Meadowview Biological Research Station, Woodford, VA, <sup>3</sup>College of Natural Resources & Environment, Virginia Tech. Longleaf pine (*Pinus palustris* P. Mill.) cannot be distinguished from the other southern pines based on wood anatomy alone. A method involving measurements of pith and second annual ring diameters, reported by Arthur Koehler in 1932, was revisited as an option for identifying longleaf pine timbers and stumps. Cross section disks of longleaf, loblolly (*P. taeda* L.), and shortleaf (*P. echinata* P. Mill.) pines were measured and the diameters of their piths and second annual rings plotted against each other. Longleaf pine could be differentiated from the other two pine species demonstrating that a method established with trees harvested more than 70 years ago is still applicable to standing timber today. No evidence was found to suggest that growth rate differences impact method applicability. In those situations where the second annual ring is intact, but not the pith, very large second annual ring diameters (> 40 mm) may provide an adaptation of the method to identify timbers with a lower probability of being longleaf pine. In addition to the identification of very old lightwood stumps as part of a longleaf pine restoration effort, both methods may be applied to timber identification in historic structures and the niche forest products industry involving the recovery and processing of highly prized longleaf pine logs from river bottoms. Measurements from relicts sampled in this study yielded results consistent with the purported range for longleaf pine in Virginia.

A CENSUS OF PURPLE PITCHER PLANT, *SARRACENIA PURPUREA* L., IN MARYLAND AND VIRGINIA. Philip M. Sheridan, Meadowview Biological Research Station, Woodford, VA. *Sarracenia purpurea* L. is a rare wetland plant in Virginia and a threatened species in Maryland, with two potential subspecies in the region. A census was performed of existing populations, all known historical data on the species compiled, reasons for the species demise investigated and dates of extinction predicted. Bloom phenology was examined to see if climate change may have influenced bloom period. Only 31% of purple pitcher plant (4 of 13) sites are extant on the western shore of Maryland and District of Columbia while 33% (14 of 42) remain in Virginia, with respective total populations of 46 and 513 clumps. Causes of regional extirpation include beaver flooding, succession, and development. Predicted pitcher plant population extinction dates, based on trend line from 130 years of data, are 2015 (Maryland) and 2055 (Virginia). Disturbance, especially natural fire, played an essential role in maintaining purple pitcher plant historically in Maryland and Virginia. *Sarracenia purpurea* blooms May 8 – June 12 in Maryland and Virginia with a peak May 18-20. Peak bloom period of *S. purpurea* may have shifted as much as a week from historical dates, perhaps due to climate change.

STUDIES OF THE HEPATICAE AND ANTHOCEROTAE ALONG HAZEL RUN IN ALUM SPRING PARK, FREDERICKSBURG, VA – IMPLICATIONS OF CLIMATE CHANGE. Stephen W. Fuller, Biology Department, University of Mary Washington, Fredericksburg, VA 22401. Climate plays a large role in determining the distribution of species, especially through physiological thresholds of tolerance to temperature and precipitation. With general warming trends, the ‘tolerance envelopes’ are expected to migrate pole-ward, and upward in elevation. Studies of the biological impact of climate change have focused on the abundance and distribution of species; changes implying impact of these abiotic factors. More reliable geographical shift information can be ascertained by studying more sedentary species, considering local extinctions and new records. Fredericksburg experienced a prolonged period with little rainfall during the last half of August and September 2010. The drying effects of this period were compounded by relatively warmer temperatures. During the fall of 2008 and 2010 exhaustive sampling was used, from the top of the stream bank to the center of the stream, within which all plant materials were surveyed for the presence of liverworts and hornworts. Despite the paucity of rain during some of the warmest months of the growing season (warmer than during the previous study in 2008), the liverworts did not seem to have suffered. There was a single place where *Conocephalum conicum* plants had dried to the point of having dead edges along the thallus. Evidently the higher temperatures and paucity of precipitation were not inhibitory to these species’ continued presence and success in this habitat.

EXAMINING POLAR AUXIN TRANSPORT EVOLUTION IN BRYOPHYTE GAMETOPHYTES. B.T. Piatkowski, G.A. Bader & D.B. Poli. Department of Biology, Roanoke College, Salem, VA 24153. Specialized cells in vascular and non-vascular plants can move auxin unidirectionally in a process termed polar auxin transport. Data collected from the liverwort, *Riccia fluitans*, suggests that simple diffusion is utilized for the transport of auxin into the cell. The hornworts, *Phaeoceros laevis* and *Anthoceros agrestis*, and the moss, *Rhizomnium* sp., displayed facilitated diffusion influx. All four species displayed facilitated diffusion as the method of auxin efflux. *Rhizomnium* showed sensitivity to the influx inhibitor 1-naphthoxyacetic acid and the efflux inhibitor 1-N-naphthylphthalamic acid suggesting that bryophyte gametophytes contain protein carriers similar to those used in PAT in vascular plants but that the process may not be unidirectional and specialized yet.

EXAMINING POLAR AUXIN TRANSPORT IN THE MOSS GAMETOPHYTE OF *DICRANUM SCOPARIUM*. Jessica N. Branning & DorothyBelle Poli. Dept. of Biol., Roanoke College, Salem VA 24153. Polar auxin transport is a process understood most in higher plants. Understanding where a physiological mechanism evolved requires examining the same process across the entire plant kingdom. Poli et al. (2003) examined polar auxin transport across the bryophyte sporophytes and learned that hornworts exhibit simple diffusion, liverworts exhibit facilitated diffusion, and mosses show active transport. However, examining polar auxin transport using traditional agar block methods has been limited in gametophytes. This

study examines the moss *Dicranium scoparium*. From Bader et al. (unpublished) it is expected that this terrestrial moss gametophyte should show facilitated diffusion. (Supported by: Jeffress Memorial Grant).

### Chemistry

DOES CO<sub>2</sub> WARM THE ENVIRONMENT? T.C. DeVore & D. Havey, Department of Chemistry and Biochemistry, MSC 4501, James Madison University, Harrisonburg VA 22807. A series of laboratory exercises have been developed that let students investigate three postulates related to global warming: 1. burning fossil fuels creates CO<sub>2</sub>, 2. CO<sub>2</sub> absorbs IR radiation, and 3. increasing the amount of CO<sub>2</sub> in the atmosphere increases the temperature of the environment. To test the first postulate, students use GC-MS and FTIR to compare the amount of CO<sub>2</sub> in a sample of automobile exhaust to the amount of CO<sub>2</sub> in ambient air. A vibrational and a rotational analysis of the spectrum for CO<sub>2</sub> and preparing a Beer's Law plot for CO<sub>2</sub> are used to test postulate 2. The Boltzmann distribution to determine the rotational temperature of the CO<sub>2</sub> in the cell is used to test postulate 3. By measuring the temperature of several different concentrations of CO<sub>2</sub>, the effect of the concentration of CO<sub>2</sub> on the temperature inside the cell is established. If all parts of this project were done, students would learn about the combustion of hydrocarbons, the IR spectra and rotational analysis of linear tri-atomic molecules, Beer's Law, the measurement of rotational temperatures based on the Boltzmann distribution and the Greenhouse Effect.

DESIGN, SYNTHESIS AND INTERACTION STUDIES OF SULFATED ALLOSTERIC MODULATORS OF THROMBIN. Preetpal Singh Sidhu, Aiye Liang, Akul Y. Mehta, May Abdel Aziz, Qibing Zhou, & Umesh R. Desai, Dept. of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA-23219. We have synthesized a library of potent, small, aromatic allosteric inhibitors of thrombin. The sulfated benzofuran scaffold was designed from the structure of sulfated lignin and contained charged, polar and hydrophobic substitutions at the 2,3,5,6 positions, which provide diversity to the library of 15 sulfated monomers and 13 sulfated dimers. Due to presence of negatively charged sulfate and carboxylate groups, these molecules are completely water soluble. Synthesis of the library of benzofurans derivatives was achieved through a multiple step, highly branched strategy, which culminated with the introduction of sulfate group(s) using microwave-assisted chemical sulfation reaction. From the library of 28 potential inhibitors, 11 inhibited thrombin with reasonable potency. Structure activity relationship studies showed sulfation at the 5-position of the benzofuran scaffold was essential for targeting thrombin. The *t*-butyl derivative was found to be the most potent with an *IC*<sub>50</sub> of 7.3 μM under physiologically relevant conditions. Michaelis-Menten kinetic studies showed that these inhibitors do not affect the affinity of chromogenic substrate for thrombin (*K*<sub>m</sub>) but greatly reduce the maximal velocity (*V*<sub>max</sub>) of reaction, indicating the allosteric mechanism of inhibition. The results of plasma clotting assays suggest that these inhibitors prolong both the

activated partial thromboplastin time and prothrombin time, of which the former is more affected in a manner similar to the heparins and hence affect the intrinsic pathway more effectively.

DEVELOPMENT OF A METHOD FOR WATER PURIFICATION UTILIZING THE ELECTROCHEMICAL PROPERTIES OF POROUS CARBON MATERIALS. Mikhail M. Goldin<sup>1</sup>, B.M. Grafov<sup>2</sup>, A.D. Davydov<sup>2</sup>, Mark M. Goldin<sup>3</sup>, & V.A. Kolesnikov<sup>4</sup>, <sup>1</sup>Dept. of Bio. and Chem., Liberty University; <sup>2</sup>Frumkin Inst. of Phys. Chem. and Electrochem; <sup>3</sup>Sklifosovsky Inst. for Emergency Medicine; <sup>4</sup>Mendeleev Univ. of Chem. Tech. of Russia. The traditional methods for decontamination of water have limitations and are relatively expensive to use. The goals of the present work were to develop a prototype electrofilter. AG-3 brand activated carbon samples were electrochemically pre-modified to initial open-circuit potentials (OCP) between -775 and +475 mV and then placed in aqueous 2.20 M *t*-butanol and 0.100 M CuSO<sub>4</sub> solutions. Concentrations were measured on an SRI Instruments 310C gas chromatograph for *t*-butanol and on a Genesys 10uv spectrometer at 808 nm for Cu<sup>2+</sup>. Effective charge transfer for each experiment was calculated using the equation  $n = (C \cdot \Delta E) / (F \cdot \Delta \Gamma)$ , where  $n$  – effective number of electrons,  $C$  (F) – differential capacitance of the sorbent electrode,  $\Delta E$  (V) – OCP change during the experiment,  $F$  (=96500 C mol<sup>-1</sup>) – Faraday's constant,  $\Delta \Gamma$  (mol) – net adsorption. The effective charge transfer for *t*-butanol ( $n < 0.1$ ) did not vary appreciably with potential, which pointed to a mechanism not involving electrooxidation or electroreduction.  $n$  for Cu<sup>2+</sup> was near 2 for potentials more negative than -470 mV and gradually decreased until +475 mV, where it reached  $n \approx 0$  indicating a major contribution from Faradaic electroreduction. Preliminary microbiological results also indicated a dependence of bacteria counts on carbon potentials. This study is supported by funds from the Center for Research and Scholarship Fund of Liberty University.

MEASUREMENT OF DOPAMINE TRANSPORTER ACTIVITY IN THE LARVAL *Drosophila* CNS USING FAST-SCAN CYCLIC VOLTAMMETRY AT CARBON-FIBER MICROELECTRODES. T. Vickery & B.J. Venton, Dept. of Chem., University of Virginia, Charlottesville, VA. *Drosophila melanogaster* is homologous to mammals in primitive neurobiology making it an advantageous model system in which to study the dynamics of dopamine regulation. Here we report a new method to measure dopamine transporter activity in isolated, *Drosophila* ventral nerve cords. A carbon fiber microelectrode is implanted into a larval ventral nerve cord, while a micropipette is simultaneously implanted approximately 10  $\mu$ m away. Dopamine is pressure ejected into the neuropil region, rich with dopaminergic neurons, and dopamine diffusion and clearance observed. Various concentrations of dopamine were applied and maximal uptake ( $V_{max}$ ) was calculated. Clearance of exogenously-applied dopamine significantly decreased in the neuropil region following treatment with 50  $\mu$ M cocaine, a dopamine transporter inhibitor. Dopamine clearance also significantly decreased in fumin mutants lacking a functional dopamine transporter. The effect of acute cocaine application on dopamine clearance is in good agreement with previous real-time, endogenous dopamine clearance measurements in *Drosophila* larvae as well as measurements made in adult

*Drosophila*. This method further validates the use of *Drosophila* as a model system to study dopamine regulation and the role of the dopamine transporter in the underlying mechanisms of drug addiction. This work was funded by NIH R01MH085159.

**SUBSTITUTION VS. ELIMINATION – THERMODYNAMICS VS. KINETICS.** Charles M. Bump, Department of Chemistry, Hampton University. The competition between substitution and elimination reactions is central to the study of organic chemistry. It is a challenge to both the classroom teacher and to the student. When reaction mechanisms are described in textbooks, generalizations are made such as “Primary alkyl halides react with strong base / nucleophiles principally by an  $S_N2$  mechanism and secondary alkyl halides react with strong base / nucleophiles primarily by an  $E2$  mechanism. Such generalizations correctly predict the reaction product and mechanism, but leave the student without a clearly defined chemical principle for the reaction path. We describe a systematic analysis of some thermodynamic and kinetic parameters of substitution and elimination reactions using the semi-empirical program MOPAC 2009. Free enthalpies of formation of starting materials and products as well as free enthalpy of formation of substitution and elimination transition states form the basis of the analysis.

**CALCULATIONAL ORGANIC CHEMISTRY – A SUPPLEMENT TO TRADITIONAL WET CHEMISTRY EXPERIMENTS.** Charles M. Bump, Edmund M. Ndip, Godson C. Nwokogu & Michelle K. Waddell, Department of Chemistry, Hampton University. The ready availability of calculational chemistry software and its widespread use in graduate education and industry presents a clarion call for calculational chemistry to be taught with other fundamental lab techniques such as distillation and recrystallization. The web site “Molecules in 4-D” ( <http://wetcbe.cmbi.ru.nl//organic//> ) provides an easy-to-use graphical interface to the MOPAC semi-empirical calculation engine with pre-defined starting materials to be edited for investigations by “users”, not just theoreticians. We will describe our record-keeping requirements, procedures, and results in using this free resource for teaching calculational chemistry with representative examples from the first and second semester of the organic chemistry sequence. We will demonstrate that calculations are fast enough that they can be conducted as real-time class demonstrations by the teacher or assigned by the teacher to small groups or individuals who report back to the class in five to ten minutes.

**O<sub>2</sub> DEPENDENCE of SINGLET OXYGEN YIELDS FROM NATURAL DISSOLVED ORGANIC MATTER (DOM).** Charles M. Sharpless, Department of Chemistry, University of Mary Washington, Fredericksburg, VA. Natural dissolved organic matter (DOM) is a heterogeneous mixture produced by decay of plant and plankton biomass. Irradiating DOM in aqueous solution produces a suite of photoreactants including singlet oxygen (<sup>1</sup>O<sub>2</sub>), superoxide (O<sub>2</sub><sup>-</sup> and its dismutation product, H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH), solvated electrons, and DOM triplet states (<sup>3</sup>DOM\*). These photoreactants can degrade aquatic pollutants and likely play a part in the conversion of DOM to inorganic carbon (i.e., CO<sub>2</sub>). Much of the chemistry involves O<sub>2</sub>, and it is important to establish how the rates of the various reactions depend on dissolved O<sub>2</sub> concentrations. Such studies can also provide



insight into the DOM photochemical mechanism, allowing determination of such properties as the excited state lifetimes of DOM precursors to various photoreactants. This talk covers relevant background and presents the results of experiments showing that DOM  $^1\text{O}_2$  quantum yields vary in approximate proportion to dissolved  $\text{O}_2$  over environmentally relevant concentrations ( $\text{DO} \sim 50$  to  $250 \mu\text{M}$ ). Modeling  $^1\text{O}_2$  production as arising from diffusion based reaction between  $^3\text{DOM}^*$  and  $\text{O}_2$  indicates that  $^3\text{DOM}^*$  has a lifetime of approximately 5 to 10 ns, regardless of the DOM source.

ALUMINUM'S CONTROL IN THE BLUING OF HYDRANGEA SEALS. Henry D. Schreiber, Andrew H. Jones, Corinne M. Lariviere, Kelly M. Mayhew, & Judith B. Cain, Department of Chemistry, Virginia Military Institute, Lexington, VA 24450. The pigment in *Hydrangea macrophylla* sepals is the anthocyanin delphinidin-3-glucoside. The color of this pigment provides a red color to the sepals, as the delphinidin-3-glucoside is in its flavylum cation structure. When grown in acidic soil, hydrangeas readily assimilate aluminum which results in blue sepals. A chemical model has been developed that attributes the blue color to  $\text{Al}^{3+}$  complexing with delphinidin-3-glucoside in its quinoidal base anion structure along with a stacked flavylum cation structure. The role of  $\text{Al}^{3+}$  is to aid in  $\text{H}^+$  transfer and act as an anchor for this stacked complex. The chemical model also predicts that the bluing plateaus once a specific excess of  $\text{Al}^{3+}$  over the delphinidin-3-glucoside is achieved. In the natural system, the color change from red-to-blue with aluminum can be duplicated spectrophotometrically by this  $\text{Al}^{3+}$ -delphinidin-3-glucoside complex without recourse to co-pigments. The threshold aluminum content in hydrangea sepals is  $40 \mu\text{g Al}^{3+}/\text{g}$  fresh sepal for bluing to occur; this threshold is relatively cultivar independent, despite the cultivar sepals having different delphinidin-3-glucoside contents. More aluminum than the threshold did not enhance the hue or intensity of the blue color of the sepal, in agreement with the chemical model.

COMBINING COMPUTATIONAL AND EXPERIMENTAL APPROACHES TO INVESTIGATE THE CATALYTIC MECHANISM OF GLYOXYSOMAL MALATE DEHYDROGENASE- PART I. H. Guterres, S. Hedrick & E. Bell. Department of Chemistry, University of Richmond, Richmond, VA 23173. Geometry optimization calculations have been used to find the minimum total energy and structure of the transition state during the conversion of malate to oxaloacetate catalysed by the enzyme glyoxysomal Malate Dehydrogenase. The proton abstraction is suggested to precede hydride transfer due to the activity of the arginine residues around the active site. To test this, two truncated models were built; model A included Histidine 220, malate and  $\text{NAD}^+$ , and model B included these three moieties from model A with the addition of three arginine residues around the active site, R124, R130, and R196. Gaussian 03W was used to build the structure of the compounds, arrange their atoms, and run the geometry optimization calculations. For model A, the result shows a single imaginary frequency that vibrates to the hydride transfer. In contrast, for model B there is a single imaginary frequency that vibrates to the proton transfer. The result suggests that the arginine residues around the active

site play important roles in facilitating the proton transfer. In silico mutation of these arginine residues is being correlated with the effects of in vitro site directed mutations to ascertain the precise roles of individual arginines in binding and catalysis.

INVESTIGATING DIRECT PHOTOLYSIS MECHANISMS OF POLYCYCLIC AROMATIC HYDROCARBONS IN NON-POLAR SOLVENTS. Jonathan S. Williams & Charles M. Sharpless, Department of Chemistry, University of Mary Washington, Fredericksburg, VA 22401 Polycyclic aromatic hydrocarbons (PAHs) are toxic components of oil spills whose fate is often controlled through photochemical reactions. PAH photolysis mechanisms in hydrophobic media are not known, hampering efforts to predict their removal rates from oil in the event of a spill. One hypothesis is that singlet oxygen ( $^1\text{O}_2$ ) generated by excited state PAHs is an important intermediate that contributes to PAH photolysis. In order to test this hypothesis, we are studying whether PAHs undergo a self-sensitized photolysis mechanism involving  $^1\text{O}_2$  in solvents of similar polarity to oil. We employed a  $^1\text{O}_2$  probe,  $\alpha$ -terpinene, to determine whether  $^1\text{O}_2$  is produced during irradiation of four PAHs: benzo[a]pyrene, benzo[e]pyrene, benz[a]anthracene, and chrysene. Loss of  $\alpha$ -terpinene in irradiated PAH mixtures revealed that  $^1\text{O}_2$  is sensitized in hexane. Additionally,  $^1\text{O}_2$  quantum yields were measured in hexane using a Stern-Völmer analysis, yielding previously unknown values for benzo[a]pyrene and benzo[e]pyrene at 0.84 and 0.70 mol  $\text{Es}^{-1}$ , respectively. To determine if this self-sensitized mechanism was occurring, we investigated whether the direct photolysis quantum yield ( $\phi_D$ ) was linearly dependent on PAH concentration as predicted by our kinetic model. Experiments showed non-linear plots of  $\phi_D$  with increasing PAH concentration, suggesting that another mechanism is contributing to PAH photolysis in non-polar media.

DETERMINATION OF AN HPLC METHOD FOR THE QUANTITATION OF ESTRADIOL AND ITS SEVEN DEGRADATION PRODUCTS. J. Chris Maxwell & Geoffrey C. Klein, Department of Molecular Biology and Chemistry, Christopher Newport University, Newport News, VA. Estradiol is an endocrine disrupting compound that mimics endogenous estrogens found in humans and can directly interact or interfere with and alter the normal growth, reproduction, or other physiological function of an organism. The need for a highly sensitive and accurate technique to measure the presence and concentrations of endocrine disrupting compounds has generated several analytical approaches for the analysis of these compounds. This work aims to determine an analytical method for the separation and quantitation of estradiol and its 7 main degradation products. The HPLC method determined to facilitate the most ideal separation used a 15-cm C16 Polar Advantage column (Dionex Corporation) with an isocratic mobile phase composed of 10% tetrahydrofuran, 63% Phosphoric Acid Dilution (0.085%), and 27% Acetonitrile at a constant flow rate of 1.5 mL/min for a total run time of 60 minutes. The detection method used was UV/Vis spectroscopy which measured absorbance at a wavelength of 225 nm. Each compound was identified individually using the developed method but peak overlap of two compounds does not support the development of a clear separation of estradiol and its degradation products. Future research will aim to

create a more complete separation where each component is indicated by a distinct non-overlapping peak and will explore the kinetics of estradiol photodegradation under various environmental conditions.

### Computer Science

**A MINIMAL WORKING CONFIGURATION FOR THE ASTERISK VOIP SERVER.** Luke D. Acree & Robert M. Marmorstein, Dept. of Computer Science, Longwood University, Farmville, VA 23909. We present a fixed-point algorithm for reducing a large set of configuration lines to a *minimal working configuration*. A *minimal working configuration* is the smallest set of configuration lines that allow an application to provide a pre-defined level of service. We apply our algorithm to the Asterisk voice-over-IP server using a baseline of one *SIP* and one analog phone connection. Using the algorithm, we are able to reduce a configuration with more than eighty files and 12, 000 lines of configuration to a simpler system containing only four files and fifty-six lines. The algorithm finds a *local minimum* configuration which, in practice, is usually the smallest working configuration set. Reducing the number of lines of configuration makes teaching and training easier and also makes it easier to customize a server to provide exactly the desired functionality without introducing security flaws.

**BIT PARALLEL SEARCH ALGORITHMS OF MOTIFS IN DNA USING TVCA.** S.V. Providence, Department of Computer Science, Hampton University, Hampton, VA 23668. Time-varying cellular automata (TVCA) are a form of cellular automata CA where the transition functions vary over time or are time dependent. The DNA motif search problem is a well-known problem in bio-informatics. Essentially it is the problem of searching for patterns in biological sequences. Two classical approaches utilize computation theory to exploit the power of deterministic finite automata (DFA). A third approach uses a filtering technique. CA is novel in motive searching. We view a CA as an aggregate of NFAs or cells where the NFA transitions are modified to account for neighboring cell states. There is a large body of literature on CA and large body of literature on NFA in motif searching. In this paper we extend work done in a previous paper by the author and the classical approach and explain the usage of a TVCA in motif searching further.

**CAN ILLEGAL AND UNETHICAL USE OF TECHNOLOGY BE EFFECTIVELY DETERRED?** Angela B. Hayden, Department of Computer Science, Hampton University, Hampton VA 23668. Modern society is advancing at such a high rate of speed that it is virtually impossible for an individual to keep up with the latest technological advancement. The worst part is the rampant disregard for ethics and ethical behavior when using technology. In an attempt to stay current with latest technology, many individuals engage in illegal and unethical practices such as illegal downloading of music or software piracy. These activities can be seen as socially acceptable behaviors. However, as computer scientist, we are well aware of the laws and policies warning against such practices. Therefore, we ask ourselves, what is the

best method for preventing illegal or unethical activity? Would the knowledge of the laws, the possible execution of a penalty, a high probability of being caught, or an enforcement of the penalty decrease the likelihood of a person engaging in illegal and unethical activities?

**ON E-MAIL REPUTATION.** Bruce C. Chittenden, Computer Science, Governor's School for Science and Technology, New Horizons Regional Education Centers, Hampton, VA 23666. Spam has been a serious problem for several years. However, over the last couple of years and more recently in the last six months the Internet Service Providers have been taking aggressive action to counter spam. These actions include the creation of Blacklists to block Spammers' IP Addresses, a Spam Button on their e-mail User-Interfaces for the user to report spam, Feedback Loops for e-mail senders so they may unsubscribe unhappy users, requiring e-mail Senders to sign their e-mails, and other aggressive measures. An entire industry has emerged around e-mail reputation with such services as creating and managing Sender Reputation Scores, maintaining Blacklists and Whitelists, reporting tools to examine Mail Exchange Server's records, tools to create the infrastructure for signed e-mails, and e-mail Service Providers that claim high deliverability rates. This paper examines the actions that should be taken to improve and maintain an e-mail reputation.

**POPULATING THE MACONACHY CUBE.** Robert A. Willis Jr. Department of Computer Science, Hampton University, Hampton, VA 23668. The purpose of this presentation is to explain the essential elements of the Maconachy Cube as seen above. The Maconachy Cube is a graphical representation of possible security scenarios one must account for in Information Assurance. Each element of the cube can be seen as representing a triplet of indices in a three dimensional array. For instance the triplet 1, 5, 2 can be seen as representing the *transmission, non repudiation, technology* triplet. The Maconachy Cube has been widely adopted as a paradigm for Information Assurance excellence, education, curricula development and for providing a "multidimensional view required to implement robust IA programs". This information assurance model covers every aspect of information security. The presentation will also outline the development of the Cube using both independent student research and a Software Development class. Keywords: Triplet, Information Assurance, Maconachy, Information States, Security Countermeasures, Security Services.

**TRUSTWORTHY COMPUTING MODEL.** Yen-Hung Hu, Department of Computer Science, Hampton University, Hampton, VA 23668. In order for a system to perform reliably, the main components of such a system must be installed and configured properly to provide appropriate operations to satisfy daily needs. These components include hardware, operating system, network protocol, application, and data. However, even though a system seems to work properly, it doesn't mean that this system is trustworthy. A trustworthy system must be able to enforce protection upon security, privacy, and usability. This research surveys and investigates several existing mechanisms to enhance trustworthiness of each component, as well as

studies their performance. A novel trustworthy computing model has been developed for enhancing trustworthiness, as well as served as the criteria to assess trustworthy computing systems.

POWER AWARE PARALLEL AND DISTRIBUTED COMPUTING USING HETEROGENEOUS SYSTEMS. S.V. Providence and A. Alexander, Department of Computer Science, Hampton University, Hampton, VA 23668. Energy efficiency is among the more important issues in supercomputing and high performance computing. We have a novel means of investigating power consumption on a cluster computer system. We examine the iozone package which is traditionally used to measure file system performance in Linux clusters and use it to give indications of power consumption proportional to file system activity. Typical method use dynamic voltage and frequency scaling which are describe in this paper. We then explain our approach and postulate its efficacy.

DISTANCE ANALYSIS OF GENE EXPRESSION PROFILE: USING GLOBAL SIGNATURES ASDIAGNOSTIC TOOLS. Lei Wang, Ganiraju Manyam, Boris Veytsman, Ancha Baranova. School of System Biology, George Mason University, Fairfax, VA. Center of Liver Diseases, Inova Fairfax Hospital, Falls Church, VA. Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA. Department of Bioinformatics & Computational Biology, The UT MD Anderson Cancer Center, Houston, TX. The traditional protein or RNA biomarkers usually suffer from either low specificity or unsatisfactory reproducibility. Here we propose a novel approach that involves measuring global distance between entire gene express profiles of normal and diseased tissues. Psoriasis, a complex autoimmune disease which pathogenesis is still not fully understood, provides an excellent model disease in which only a portion of a patient's skin will be affected, making it possible to study the difference between lesions, uninvolved skin from patients and healthy skin from non-diseased controls. To address the performance of global distances as biomarkers, we analyzed the data generated by microarray experiments and used Mahalanobis distance as an estimation of the degree of differentiation. The effectiveness of global signatures was compared with that of traditional specific signatures, which are defined as highest ranked differentially expressed genes. We demonstrated that global distances of gene expression profiles can serve as reliable good classifiers, and therefore it is worth considering to use them as diagnostic tools.

## Education

SERVICE-LEARNING IN ENVIRONMENTAL STUDIES: THREE SUCCESSFUL APPROACHES FOR UNDERGRADUATE EDUCATION. Woodward S. Bousquet, Environmental Studies and Biology Departments, Shenandoah University, Winchester, VA 22601. Over the past three decades, service-learning in higher education has expanded considerably. More high school students arrive with service experience, service is built into undergraduate courses,

and it is added to university mission statements and graduation requirements. However, to be effective, service-learning must not only meet a community's needs. It must also enhance student learning, advance the university's mission and match students' needs and skills. Three successful service-learning approaches used in Shenandoah University's environmental studies courses since 1994 involve preparing booklets and brochures, teaching through teaching stations, and forming research partnerships. Booklets and brochures have a lasting impact and give university students a tangible product to show others. Dividing a field trip for school children into separate teaching stations cuts down on planning time and helps university students hone their teaching techniques. Forming research partnerships with local nongovernmental organizations and government agencies can connect students' service with actual community needs and provide ongoing opportunities for future classes. All three of these approaches match student and institutional goals, fit the structure of their respective courses, and sustain themselves in the curriculum by building upon past projects' accomplishments.

SCIENCE ON A SPHERE® AT THE SCIENCE MUSEUM OF VIRGINIA. E. G. Maurakis, R. Conti, E. Alford, D. Olli. Science Museum of Virginia, 2500 W. Broad Street, Richmond, VA 23220. Objectives of this program are to use the interactive six ft diameter sphere and associated visualizations to educate the public about climate change using inquiry based methods, and evaluate program outcomes. The program focuses on the National Oceanic and Atmospheric Administration's (NOAA) learning goals in climate literacy: the sun is the primary source for earth's climate system, climate is regulated by complex interactions among components of earth systems; life on earth depends on, affects, and is shaped by climate, climate varies over space and time through natural and human-made processes, and human activities are impacting the climate system. Key messages are climate change is occurring, detrimental aspects are greater than beneficial aspects, humans are causing climate change, and humans can solve the problem. Funded by NOAA award NA10SEC0080023 and Science Museum of Virginia.

ON THE USE OF TECHNOLOGIES SUCH AS ALICE, GREENFOOT, AND SCRATCH TO TEACH COMPUTER SCIENCE TO HIGH SCHOOL STUDENTS. Bruce C. Chittenden, Computer Science, Governor's School for Science and Technology, New Horizons Regional Education Centers, 520 Butler Farm Road, Hampton, VA 23666. While all of these technologies are very effective at introducing the student to Computer Science each has its own strengths and weaknesses and the technology needs to be chosen very carefully based upon several factors. This paper examines the original design objectives of each of the technologies, the Computer Science concepts that these technologies present, and how these technologies are actually being used in the classrooms.

APPLICATION OF 21<sup>ST</sup> CENTURY LEARNING AND INNOVATION SKILLS AT THE SCIENCE MUSEUM OF VIRGINIA. E. G. Maurakis, E. Alford, R. Conti, E. Voelkel, R. Shires, and D. Smith. Science Museum of Virginia, 2500 W. Broad Street, Richmond, VA 23220. Objectives of this program are to examine the delivery of science, technology, engineering, and mathematics (STEM) information

using 21<sup>st</sup> century learning and innovation skills proposed by the Institute of Museums and Library Services (IMLS) in programs and exhibitions at the science museum. IMLS's skills being implemented and evaluated are critical thinking and problem solving, creativity and innovation, communication and collaboration, visual literacy, scientific and numerical literacy, cross disciplinary thinking, and basic literacy. Laboratory experiences (e.g. EcoLab), exhibitions (i.e., Science Unplugged), and programs (e.g. Summer STEM workshops) were evaluated using IMLS 21<sup>st</sup> century learning and innovation skills, meeting most of the IMLS skill categories.

AN INFORMAL SCIENCE APPROACH FOR RENEWABLE AND NONRENEWABLE ENERGY CONCEPTS. David B.Hagan, Science Museum of Virginia, 2500 W. Broad Street, Richmond, VA 23220. The Science Museum of Virginia is a center of informal science education, generally defined as "science teaching and learning that occurs outside of the formal school curriculum in places such as museums, the media, and community-based programs." (NSTA Position Statement: Informal Science Education). The museum's project presents components in several forms of media focused on alternative and conventional energy sources. These include *Question Power*, an original six-minute HD video presenting the challenge of discovering clean, abundant energy sources, *Watt Wall*, a large computer-driven museum wall display showing energy demand, and a set of K-12 curriculum materials. The curriculum materials include a 2' x 3' original detailed map of Virginia showing sites of sources of energy and delivery mechanisms, including alternative and conventional sources. The curriculum materials challenge common misconceptions about energy sources. Examples include: "Wind and solar energy will provide all energy needs in the near future", and "Conventional sources of energy will last indefinitely." The underlying premise of the K-12 materials is that every energy source has advantages and disadvantages. With increasing global demand, currently known clean sources of energy cannot be expected to serve the world's needs using present technology in coming decades. The search for solutions for the future lies with future generations.

BIODIVERSITY AND WATER QUALITY INVESTIGATIONS IN SCHOOLYARD MESOCOSMS. H. S. Houtz<sup>1</sup>, A. B. Wright<sup>2</sup> & S. E. Marwitz<sup>3</sup>, VCU Life Sciences<sup>1</sup>, VCU Dept. of Biology<sup>2</sup>, and Maggie L. Walker Governor's School<sup>3</sup>. Mesocosms are experimental water enclosures that support naturally occurring aquatic communities. Because they provide an easy means to manipulate variables, mesocosm arrays have been used since the early 1960's to address a variety of scientific questions. Although mesocosm research has had a long history, mesocosms are an underutilized teaching tool that can provide a practical way for school children to design and implement simple scientific investigations within school grounds. VCU's Environmental Outreach Education team has placed mesocosms in diverse habitats (wetland, forest, open field, etc.) at VCU's Rice Center and at 7 Richmond area public schools. Over the past 3 years, high school and middle school students have measured water quality and monitored colonization and development of macroinvertebrate and algal communities.

Ongoing student research projects aim to investigate the effects of canopy cover, habitat isolation, and nutrient additions on community structure. Data have been shared and analyzed collaboratively among the school systems. Biodiversity and water quality have varied greatly seasonally and spatially among the sampling locations, which range from inner-city Richmond to Charles City County, Virginia. This project was made possible by the Howard Hughes Medical Institute (HHMI).

### Environmental Science

MODELING FISH SPECIES RICHNESS AND DIVERSITY IN LOWER PIEDMONT STREAMS. E. G. Maurakis<sup>1,2</sup>, D. V. Grimes<sup>3</sup>, A. Schutt<sup>4</sup>, and S. Short.<sup>1</sup> <sup>1</sup>Science Museum of Virginia, <sup>2</sup>University of Richmond, <sup>3</sup>Virginia Department of Environmental Quality, <sup>4</sup>VCU. Objectives were to model fish species richness and diversity using biotic and abiotic environmental factors in Quantico Creek (a forested watershed) and Cameron Run (an urban watershed), Virginia. A total of 17 physical, chemical, and biological factors were used to construct mathematical models to predict fish species richness and diversity in each watershed. Fish species richness in Quantico Creek can be predicted using season, stream order, elevation, river km, stream width and depth, watershed size and percent undeveloped land cover. Fish species richness in Cameron Run can be predicted with stream gradient, stream flow, water temperature, and percent undeveloped land cover. Funded by U.S. Department of Energy Grant DE-FG02-08ER64625.

PRELIMINARY ANALYSIS OF BAY FILTER UNIT SUCCESS IN FREDERICKSBURG, VA. MICHAEL L. BASS, UNIVERSITY OF MARY WASHINGTON, FREDERICKSBURG, VA. Virginia Department of Conservation and Recreation (DCR) evaluates and approves manufactured treatment devices (MTD's) deemed reasonable methods of prevention, control and/or treatment of storm water runoff. Virginia's stormwater management programs are implemented under: Virginia Stormwater Management Law and Virginia Stormwater Management Regulations. DCR maintains the authority to regulate BMP methods used in Virginia to control stormwater runoff under the Virginia Technology Assessment Protocol (VTAP). The assessment protocol deals with the MTD's that are designed for, reducing stormwater runoff volume, reducing peak runoff rate and/or and reducing total phosphorous (TP). The goal of the VTAP regarding runoff quality control is to determine how much a specific MTD can remove total phosphorous (TP). MTD's seeking certification for runoff quality control in Virginia will only be approved for TP removal at this time, requiring 50% TP removal for influent with TP concentrations ranging from 0.15 mg/L to 0.5 mg/L. Additional requirements are 80% removal of TSS for influent with TSS concentrations ranging from 100 mg/L to 200 mg/L and > 80% removal of TSS for influent with concentrations greater than 200 mg/L. Baysaver Technologies, Inc. has applied for interim approvals to use the Bay Filter System to meet Virginia requirements for treating stormwater runoff. Flow through the filter system in gravity-driven and self-regulating. The monitoring program is intended to demonstrate through field testing that Bay Filter is capable of



removing contaminants from stormwater runoff. The field test will demonstrate the removal efficiencies attained by the system for TSS, TP, Cu, Zn and other pollutants. This will then be used to confirm that the system meets stormwater regulations which require the removal of a minimum 80% of the total suspended sediment load and treatment of nutrients to the maximum extent feasible. The field testing program will collect discrete samples from the influent and effluent of the BayFilter. These samples will be analyzed using standard EPA protocols for total suspended solids (TSS), particle size distribution (PSD), nutrients as well as metal concentrations. Removal efficiencies will be calculated based on this data using standard scientific methods. Precipitation and flow records will be acquired during these events as well. The testing program is anticipated to take 12-18 months to complete and will include at least 15 qualifying storm events. BayFilter systems to be monitored will treat the stormwater runoff from Trinity Episcopal Church property in Fredericksburg, VA. Stormwater runoff from the paved area transports dissolved, colloidal, suspended and settleable solids in a heterogeneous mixture, which includes metals, organic compounds and nutrients. These constituents result from atmospheric deposition, traffic activities, vehicular wear, pavement degradation and deicing, landscape maintenance and littering. The nutrient load from the site is expected to vary seasonally.

ASSESSING WATER QUALITY OF CEDAR CREEK USING BENTHIC MACROINVERTEBRATES. John V. Stevens, Briana L. Barron, James E. Bisset, Linden E. Lewis, Daniel A. Milhon, Cory M. Miller, Benjamin S. Sawyer, Amy L. Smith & Woodward S. Bousquet, Environmental Studies Department, Shenandoah University, Winchester Virginia 22601. In the spring and fall of 2010, Shenandoah University researchers assessed the water quality of Cedar Creek in Frederick and Shenandoah Counties, Virginia. They used the Environmental Protection Agency's Rapid Bioassessment Protocols (RBPs) to select sampling sites and collect specimens. In addition to choosing three representative sites on the creek's main stem, the researchers selected two locations on ecologically distinct tributaries: Paddy Run (a cold-water montane stream), and Meadow Brook (a warm-water valley stream). Analysis was performed with the Virginia Stream Condition Index (VSCI), a multimetric measure based on the diversity, pollution tolerance and feeding categories of the invertebrates collected. A majority of the sites had a VSCI water quality score of >73, placing them in the excellent category. Only Meadow Brook was rated as severely impaired (avg. VSCI score = 32.2), most likely due to surrounding agricultural and residential development. These findings affirm previous studies that rated Cedar Creek's overall water quality as among the best in the Shenandoah Valley region, using chemical, physical and fish community data. Best management practices should be implemented to protect this beautiful, high-quality watershed.

## Medical Science

THE ROLE OF  $\alpha 5$  NICOTINIC ACETYLCHOLINE RECEPTORS IN ACUTE AND CHRONIC ALCOHOL BEHAVIORS IN MICE. Anton J. Dawson & M. Imad

Damaj. Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. The high incidence of nicotine dependence in alcoholics suggests that nicotinic acetylcholine receptors (nAChRs) may be involved in both nicotine and alcohol dependence. Genetic studies suggest that the  $\alpha 5$  nAChR-coding gene is significantly associated in both nicotine and alcohol dependence phenotypes, but virtually no studies have investigated this receptor subtype with regard to alcohol's effects *in vivo*. We hypothesized that the  $\alpha 5$  nAChRs mediate some acute and chronic behavioral effects of alcohol in alcohol preferring C57BL/6J mice lacking  $\alpha 5$  gene expression ( $\alpha 5$  KO mice). We tested their response to alcohol's acute effects including initial sensitivity and functional tolerance through the loss of righting reflex assay, anxiolysis using elevated plus maze, hypothermia, and locomotor depression. For chronic effects, we chose to study alcohol intake and preference using two-bottle choice and aspects of alcohol reward using conditioned place preference. Our results showed that  $\alpha 5$  KO mice, indeed respond differentially to acute and chronic ethanol exposure. These data suggest that  $\alpha 5$  nAChRs may exert differential effects on the acute and chronic effects of alcohol and may, therefore, represent a potential therapeutic target for the treatment of alcohol and nicotine co-abuse in the future.

FACTORS RELATED WITH ACTIVE LIVING FOR INDIVIDUALS IN THE WESTERN REGION OF USA. S. Tiraphat, Q. Zhang, J.G. Behr, & L. Shepherd. Old Dominion University, Norfolk, VA 23529. Adequate physical activity is a major contributor to a healthy living and prolonging life. Among extreme geographic diversity of the US, the disparity in physical activity (PA) has remained substantially across the region, with the Western part having the highest activity prevalence. It is a challenge to study and identify what factors influence on active lifestyle of people in the Western area. Objective: this study is to investigate the influences of individual and environmental contexts on the odd of being physically active of people in the Western region. Method: data from 2007 BRFSS is obtained and linked with external environmental data sources from US Census Bureau, USDA, and NORSIS databases. A two-level logistic regression is analyzed. Outcome variable is meeting PA recommendation according to CDC guideline (yes / no). Individual-level variables include demographic factors, socio-economic conditions, lifestyle, and health status. Environmental -level variables as a county scale include physical activity built environments (local facilities, green area, and outdoor activity resources), educational context, crime rate, and natural amenity. Results: At the individual level, White, male, consuming more fruits and vegetables, more satisfied with life, higher income, higher education, better health, and younger are significantly positive with the meeting PA recommendation. At the county level, outdoor activity resources and educational context are the significant predictors of the meeting PA. This study emphasizes the advantage of built environments especially outdoor activity resources on active living of people in the Western part.

REGIONAL GENE EXPRESSION CHANGES ASSOCIATED WITH INCREASED VOLUNTARY ETHANOL CONSUMPTION DUE TO DEPRIVATION IN C57BL/6J AND C57BL/6NCRL MICE. J.A. Warner, R.T. Khisti & M.F. Miles, Virginia Commonwealth University. The alcohol deprivation

effect (ADE) is an increase in ethanol consumption after abstinence and is considered a rodent model of relapse in human alcoholics. This study used microarray analysis to identify changes in gene expression in nucleus accumbens, prefrontal cortex, and ventral midbrain of C57BL/6NCrl mice after ethanol deprivation. Nearly 800 mRNA transcripts were significantly regulated, including several primary targets of ethanol, with most regulation in NAC. Analysis revealed over-represented biological processes, cell signaling pathways, and transcription factor binding sites, as well as novel coordinately regulated gene networks, with the common theme of neuroplasticity. Areas highly represented included calcium, sodium, potassium, and chloride currents, transcription and translation, neuronal remodeling, and control of cell fate. Components of GABA, glutamate, opioid and serotonin systems were regulated. Binding sites for diverse transcription factors, including CREB, were over-represented. Novel gene networks were identified related to gene expression, protein trafficking, and cellular function and maintenance. Regulated genes likely alter neuronal function by modulating excitability, protein turnover, and connectivity, and represent novel targets for further development of pharmacological and genetic therapies for relapse in alcoholism.

THE CENTRAL MECHANISMS OF GASTRIN-RELEASING PEPTIDE-INDUCED ANOREXIA. C. R. Dougherty & M. A. Cline. Dept. of Biology, Radford University, Radford, VA 24142. Recently our laboratory demonstrated that intracerebroventricular (ICV) injection of gastrin-releasing peptide (GRP), a member of the bombesin family, caused primary anorexigenic effect in broiler chicks. Currently the central mechanisms behind the anorexigenic effect of GRP are poorly understood. We hypothesized that this effect is likely mediated through similar neurotransmitters that are known to be involved in hypothalamic appetite regulation. c-Fos immunoreactivity was used in order to visualize changes in neuronal activity in regions primarily associated with satiety and appetite regulation as a result of ICV administration of GRP. We are currently in the process of further studying the central mechanisms of GRP via real-time PCR. Further investigation into the central mechanisms of GRP-induced anorexia could have significant implications in the management of obesity if pharmaceutical applications are pursued.

OPIOID AND HIV-1 ASSOCIATED NEURODEGENERATION: POSSIBLE REGULATION BY THE P2X<sub>4</sub> RECEPTOR IN PRIMARY MOUSE STRIATAL CELLS. M.E. Sorrell, S. Zou, P.E. Knapp, and K.F. Hauser, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298. HIV-1-associated neurocognitive disorders (HAND) are seen in up to 50% of AIDS patients and individuals who abuse opiates can have an increased incidence of HAND with more severe symptoms. Microglia play a role in HIV-1 neuropathogenesis since activation produces cytokines, chemokines, and other inflammatory molecules that can lead to neuronal injury and death. Recently, morphine has been reported to increase microglial motility by modifying P2X<sub>4</sub> signaling. This is important because mu opioid receptor agonists can increase HIV-1 replication, potentiate the release of oxyradicals and glutamate, and increase cytokine production in HIV-1 Tat-exposed microglia. To examine whether HIV-1 and/or

opioid-induced microglial activation and neurotoxicity are mediated via purinergic signaling, co-cultures of primary neurons and mixed glia from mouse striatum were treated with vehicle, Tat and/or morphine  $\pm$  TNP-ATP, which blocks P2X<sub>1-7</sub> receptors. Individual neurons were repeatedly tracked for 72 hours and neuron survival vs. time was assessed. In neurons co-exposed to Tat  $\pm$  morphine, treatment with TNP-ATP significantly blocked Tat-mediated neurotoxicity. Further neuron survival studies showed that TNP-ATP dose dependently prevented Tat and morphine toxicity, both when treated in combination and separately. Our data implicates that the activation of P2X receptors are critical in opiate and HIV neurotoxicity, while further work needs to be done to verify the role of the P2X<sub>4</sub> receptor in our HIV and opioid model.

THE IMPACT OF ADOLESCENT NICOTINE EXPOSURE ON DRUG DEPENDENCE IN ADULTHOOD. Mai Alajaji, M.S. & M. Imad Damaj, Ph.D. Department of Pharmacology & Toxicology Virginia Commonwealth University, Richmond, VA 23298. Smoking among adolescents is a strong predictor of future drug abuse and dependence in adulthood. A number of studies have suggested that adolescents pre-exposed to nicotine may suffer permanent disruption of the brain's reward systems through changes in dopamine receptor function. We hypothesize that nicotine exposure during adolescence causes long lasting neurobiological alterations that increase the likelihood of cocaine use in adulthood. Conditioned place preference data showed that a 7-day exposure to 0.5 mg/kg of nicotine altered cocaine-induced responses. In contrast, neither 1 day exposure nor a low dose of nicotine (0.1 mg/kg) elicited this effect. A follow-up study was undertaken to determine if this enhancement generally applies to other drugs of abuse. Pre-exposure to 0.5 mg/kg nicotine during early adolescence demonstrated significant enhancement to d-amphetamine and morphine preference in a CPP model. Similar to the effects seen with reward, exposure of early adolescent mice to nicotine also enhanced acute locomotor activity and locomotor sensitization to cocaine in adulthood. Our data strongly suggest that nicotine intake during adolescence may act to cross-sensitize the brain to cocaine's long-term changes in the brain. Further research will be required in order to more fully examine the mechanisms of action for the observed changes in cocaine rewards.

CHANGES IN GENE EXPRESSION DURING IMCD3 CELL DIFFERENTIATION. Brittany N. Abbatiello & Deborah L. Zies, Dept. of Biol. Sci., Univ. of Mary Washington, Fredericksburg, VA. Renal Cell Carcinoma (RCC) accounts for 90% of kidney cancer cases and is extremely difficult to treat. Understanding the mechanisms by which renal cells become de-differentiated during the transformation process is an important step in understanding this deadly disease. Identification of the genes involved in kidney cell differentiation may lead to a better understanding of the de-differentiation process. Therefore, the goal of this study is to identify genes involved in the differentiation of kidney cells. We determined growth conditions for undifferentiated and differentiated IMCD3 cells. Cells were plated into 6 well plates and duplicate wells were lysed each day for 9 days. RNA was collected, converted to cDNA, and QPCR was performed for Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) and Epithelial sodium channel subunit alpha

(ENaCa). GAPDH expression did not change over time. IMCD3 cells were determined to be differentiated when QPCR results correlated with expected increase in expression of ENaCa. The RNA from undifferentiated samples (day 4) and differentiated samples (day 7) were compared in a microarray experiment. Bioinformatic analysis of the results identified four candidate genes, neuronal guanine nucleotide exchange factor (NGEF), roundabout homolog 1 (ROBO1), transforming growth factor 1 beta (TGFB1), and brain derived neurotropic factor (BDNF). These genes differ in expression between undifferentiated and differentiated cells and have functions known to be associated with development or differentiation. Future studies will characterize these genes to determine if they play a role in cell differentiation or cancer cell de-differentiation.

ASPECTS OF ETHANOL RESPONSE IN *C. ELEGANS*. J. Alaimo, A. Davies & J. Bettinger, Pharm/Tox, VCU, Richmond VA 23298. Alcohol abuse is a disorder with a poorly understood etiology that includes both genetic and environmental influences. One factor found to influence drinking behavior is variation in genes encoding ethanol metabolism machinery. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are two major enzymes involved in the ethanol metabolic pathway. We directly tested the effects of altering the function of these enzymes on ethanol responsive behaviors in the nematode, *C. elegans*. Through homology searches we have identified two ADH-like enzymes that are encoded by *sodh-1* and *H24K24.3*. We have found that at 200mM and 400mM exogenous ethanol, worms lacking SODH-1 are hypersensitive to the initial effects of ethanol relative to wild type, but knockdown of *H24K24.3* caused no significant behavioral changes at either dose. We tested if this sensitivity to ethanol reflected an increase in internal ethanol concentrations and found that tissue concentration is increased in *sodh-1* mutants, but not in *H24K24.3* mutants. Moreover, *sodh-1* and *H24K24.3* mutants develop acute tolerance to ethanol suggesting that these components of the alcohol metabolism machinery are dispensable for this process. The *C. elegans* genome encodes 13 ALDH enzymes that are highly conserved between worms and humans. We have found that knockdown of either *alh-6* or *alh-13* results in altered ethanol sensitivity at 400mM exogenous ethanol, but internal tissue concentrations trend towards an increase relative to wild type. This suggests that the lack of ALDH function may cause a buildup of acetaldehyde, which would be converted by ADH into ethanol. Collectively, we find that altered ethanol metabolism in worms results in a mild effect on ethanol response behaviors.

GENE NETWORKS OF ALCOHOL DEPENDENCE. S.P. Farris, K.L. Shelton, & M.F. Miles. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. The molecular mechanisms underlying alcohol abuse and alcoholism are largely unknown, however, divergent patterns in gene expression are critical molecular endophenotypes regulating the risk of developing alcohol dependence. Using a mouse model of intermittent ethanol self-administration we have conducted Weighted Gene Co-expression Network Analysis (WGCNA) of medial prefrontal cortex (PFC) to identify gene expression networks associated with ethanol intake. WGCNA revealed a significant correlation of ethanol intake to myelin-associated gene expression. Prior work from our laboratory and others' have

identified decreased expression of myelin in the PFC of alcoholics. Additionally, our laboratory has shown myelin gene expression may be a latent factor influencing acute ethanol sensitivity which influences long-term drinking behavior. Therefore, we hypothesized a myelin network functions in acute ethanol behavioral sensitivity. Using the cuprizone model of demyelination in C57BL/6J male mice we determined that differential expression of a myelin gene network impacts the duration for the loss of righting reflex, a phenotypic measure of acute ethanol behavioral sensitivity. Cuprizone treated mice and controls exhibited no difference in blood ethanol concentrations, suggesting the phenotypic impact of ethanol administration is a pharmacodynamic effect of myelin and not due to non-specific effects on the pharmacokinetics of ethanol. Our results suggest myelin may be a molecular endophenotype influencing acute behavioral sensitivity and the risk of developing alcohol dependence. Supported by NIAAA grants U01 AA016667 to MFM and F31 AA018615 to SPF.

GENE EXPRESSION OF GASTRIC CYTOKINES AND HORMONES IN OBESITY ASSOCIATED LIVER INFLAMMATION. R. Mehta<sup>1,2</sup>, A. Birerdinc<sup>1,2</sup>, L. Wang<sup>1,2</sup>, N. Hossain<sup>2,3</sup>, A. Afendi<sup>2,3</sup>, V. Chandhoke<sup>1,2</sup>, Z. Younossi<sup>1,2,3</sup> & A. Baranova<sup>1,2</sup> <sup>1</sup>Molecular and Microbiology Department and Center for the Study of Genomics in Liver Diseases, George Mason University, Fairfax, VA, USA.

<sup>2</sup>Translational Research Institute, Inova Health System, Falls Church, VA, USA.

<sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA, USA.

Obesity is one of fastest growing global epidemics. Obesity is a complex multisystem disease resulting from a failure in the normal homeostatic mechanisms which regulates food intake, fat storage, and energy utilization. Further in obesity, there is interplay of substances produced by various organs, including visceral adipose, liver, muscle and gastric tissues. Obesity is strongly associated with numerous degenerative conditions, particularly non-alcoholic fatty liver disease (NAFLD). Consequently, deregulation of genes involved in energy homeostasis and expenditure may play an important role in not only obesity, but also the pathogenesis of the diseases stemming from this condition. Soluble molecules produced by gastric tissues and their receptors may play a role in the pathogenesis of obesity-related NAFLD. In this study, we assessed the association of gastric tissue gene expression of obesity related genes and inflammatory cytokines with NAFLD in obese patients. We demonstrate a correlation of gastric CNR1 and cytokines with hepatic inflammation. This data indicates that gastric tissue may play a previously unexplored role in the pathogenesis of obesity associated NAFLD. With further validation in a larger cohort, the differentially expressed genes detected in this study may provide novel mechanistic insights into the pathology of NAFLD.

DYNAMICS OF SUBSTRATE INTERACTIONS IN tRNA (m<sup>1</sup>G37) METHYLTRANSFERASE: IMPLICATIONS FOR DRUG DISCOVERY. M. K. Palesis<sup>1</sup>, J. C. Hackett<sup>2</sup>, & W.M. Holmes<sup>3</sup>, Departments of <sup>1</sup>Pharmacology and Toxicology, <sup>2</sup>Medicinal Chemistry, and <sup>3</sup>Microbiology, Virginia Commonwealth University, Richmond, VA 23298. The bacterial enzyme t-RNA (m<sup>1</sup>G37) methyltransferase (TrmD), a potential antimicrobial drug target, methylates the G37 nucleotide of tRNA using S-adenosyl methionine (SAM) as the methyl donor. This

methylation reduces the occurrence of frame shift mutations during protein translation. Molecular dynamics simulations of TrmD implicate secondary structural elements which may contribute to active site accessibility. Substrate-enzyme interactions were studied using Isothermal Titration Calorimetry (ITC). Molecular dynamics simulations together with ITC data suggest cooperativity between two active sites on the enzyme. To further investigate this cooperativity, a series of experiments have been initiated. Because SAM is a ubiquitous substrate for many different methyltransferases in the cell, analogs of this ligand may not be suitable for drug development. It is therefore important to understand the structural elements involved in the mechanism of action which will subsequently enable a more targeted approach to the development of new antimicrobial drugs.

COMBINING CANNABINOIDS AND RADIATION THERAPY IN BREAST CANCER. S.M. Emery, E. Sumner, A. Lichtman & D.A. Gewirtz, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Numerous reports have shown that in several preclinical models cannabinoids have the potential for improving the effectiveness of cancer treatment. Given that cannabinoids are already used as a palliative treatment during cancer therapy, we investigated whether select agents could enhance the effectiveness of radiation, which is used in treatment of breast cancer. We assessed the impact of Win55, 212-2 (Win2), a full efficacy cannabinoid agonist, as well as Cannabidiol (CBD) and  $\Delta^9$ -Tetrahydrocannabinol (THC) in combination with radiation in MCF-7 (human p53 wild type), MDA-MB-231 (human p53 mutant), and 4T1 (mouse p53 null) breast tumor cell lines as well as in MCF-10a cells, a human non-transformed cell line. Win2 significantly enhanced the antiproliferative actions of radiation (2 Gy) in MCF-7, MDA-MB-231 cells as well as 4T1 cells but not in MCF-10a cells. Unexpectedly, CBD and THC did not produce the same effect as Win2, suggesting differences between the compound classes. We also were able to show in MCF-7 cells that when using Win55,212-3 the inactive enantiomer of Win2 there was no effect alone as well as no enhancement with the combination. Our data indicates that Win2 (but not THC) has the potential to enhance current radiation therapy used against breast cancer.

miRNAs AND THEIR TARGET GENE EXPRESSION IN THE VISCERAL ADIPOSE TISSUE OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) PATIENTS. H. Sharma<sup>1,2</sup>, M. Estep<sup>1</sup>, N. Hossain<sup>1</sup>, H. Elarainy<sup>1</sup>, Z. Goodman<sup>2,3</sup>, V. Chandhoke<sup>2</sup>, A. Baranova<sup>2,3</sup>, Z. Younossi<sup>1, 2, 3</sup>, <sup>1</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA, <sup>2</sup>School of Systems Biology, George Mason University, Manassas, VA and <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA. There are many biological molecules involved in the pathogenesis of Non-Alcoholic Steatohepatitis (NASH) and its progression from NAFLD. miRNAs are known to inhibit the translation of their target mRNA. At least 113 species of miRNAs are differentially expressed in patients with NASH within visceral adipose. In this study, we set out to measure the differential levels of expression for six miRNA target genes and the components of the miRNA processing apparatus in patients with simple steatosis vs NASH. We discovered that while in NASH patients expression levels increase for only two

pre-processed miRNAs, for miRNA processing enzymes mRNA level increase throughout the pathway. When the expression levels of several NASH-related genes were studied, we observed concerted changes in the expression levels of the target genes (increased) and levels of miRNA responsible for regulation of these genes (decreased). This observation suggests that regulation by miRNA may be involved in the pathogenesis of NASH. These results warrant additional research in order to establish the precise role of the miRNA regulated genes and their miRNA regulators.

THE ROLE OF CCR5 IN HIV-1 TAT AND MORPHINE MEDIATED NEURODEGENERATION. E.M. Podhaizer<sup>1</sup>, Y. Zhang<sup>2</sup>, P.E. Knapp<sup>1,3</sup>, & K.F. Hauser<sup>1</sup>, Depts. of <sup>1</sup>Pharmacology & Toxicology, <sup>2</sup>Medicinal Chemistry, <sup>3</sup>Anatomy & Neurobiology, Virginia Commonwealth University, Richmond VA, 23298. Injection drug use, such as heroin abuse, is closely linked to both the spread and pathogenesis of HIV-1. The effects of HIV-1 Tat, in models of HIV-1 infection, have been shown to be worsened with the addition of morphine and include measures of neurotoxicity, dendritic pathology, and astrocyte calcium and cytokine/chemokine release. CCR5 has been suggested to be involved in Tat-induced signaling through Tat's effects on pro-inflammatory CCR5 ligand secretion. Additionally, morphine and the mu opioid receptor have been shown to regulate CCR5 ligand secretion and both receptor expression and protein levels. Thus, it was hypothesized that inhibition of CCR5 would be protective against neurodegeneration and glial inflammatory signaling. Neurotoxicity was examined in neuronal-mixed glial co-cultures treated with morphine (500 nM), Tat (100 nM), or the combination, in the presence or absence of the CCR5 antagonist maraviroc (50 nM). Maraviroc pretreatment prevented the synergistic neurotoxic effects of collective Tat and morphine treatment. CCR5 inhibition in cultures heavily enriched in astrocytes suppressed both RANTES and MCP-1 chemokine production, but had no effect on TNF- $\alpha$ . This data suggests that CCR5 is a critical convergence point of Tat and morphine signaling. *Supported by NIH P01 DA019398 & T32 DA007027.*

NADPH OXIDASE-MEDIATED INFLAMMASOME ACTIVATION BY ELEVATED HOMOCYSTEINE IN MOUSE PODOCYTES AND GLOMERULI. J. M. Abais, C. Zhang, M. Xia, K. Boini, L. A. Laperle, A. M. Thacker & P-L. Li, Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Hyperhomocysteinemia (hHcys) is an important pathogenic factor contributing to the progression of end-stage renal disease. Recent studies in our laboratory have found that Hcys can activate the inflammasome, potentially turning on the inflammatory response. Hcys is also known to activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and this present study was designed to test whether NADPH oxidase-mediated redox signaling contributes to Hcys-induced inflammasome activation in podocytes. Confocal microscopy showed that Hcys caused a 2.5-fold increase in colocalization between the 3 major inflammasome components - NALP3, apoptosis-associated speck-like protein (ASC), and caspase-1. Furthermore, Hcys increased caspase-1 activity, IL-1 $\beta$ , and superoxide production, and all of these changes in podocytes were significantly blocked by inhibition of NADPH oxidase using apocynin, gp91<sup>phox</sup> peptide, and gp91<sup>phox</sup> siRNA. *In vivo*, gp91<sup>phox</sup> knockout mice protected against inflammasome



formation and dysfunction induced by hHcys. These results suggest that Hcys-induced NADPH oxidase activation is importantly involved in turning on NALP3 inflammasomes, making both NADPH oxidase and the inflammasome potential therapeutic targets for preventing Hcys-induced glomerular sclerosis.

**ROLE OF ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR IN A MURINE DEXTRAN SULFATE SODIUM-INDUCED COLITIS MODEL.** S. AlSharari, H. Akbarali, & M.I. Damaj. Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Substantial evidence suggests an association between cigarette smoking and the incidence and severity of ulcerative colitis (UC), a common human inflammatory bowel disease (IBD). Nicotine seems to be a key mediator of this response as has been demonstrated by the use of transdermal patches and nicotine enemas. The mechanism through which nicotine acts is not well understood. Identification of nAChR subtypes and their role in UC would lead to better understanding of the disease and the development of better pharmacological treatment of UC. We investigated the role of  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 7$  and  $\beta 2$  nAChR subtypes in a murine model of UC in which dextran sulphate sodium (DSS) to induce colitis in C57 mice and measured a Disease Activity Index (DAI). In addition,  $\alpha 7$  KO mice displayed a significantly increased in DAI value (diarrhea, irritation around the anal area, hematochezia, and loss of body weight) compared to their WT littermates. Furthermore, treatment with PHA-543613, a selective  $\alpha 7$  agonist, significantly reduced DAI signs in DSS-treated mice compare with vehicle DSS-treated mice. Moreover, we use PNU-120596, a positive allosteric modulator, and Choline chloride, an  $\alpha 7$  nAChR full agonist, were both reduced DAI signs in DSS-treated mice. These results suggest that  $\alpha 7$  nAChR has a protective role in colitis.

**THE ROLE OF 2-AG ENDOCANNABINOID NEUROTRANSMISSION IN NICOTINE REWARD AND WITHDRAWAL.** P. Muldoon, A. Lichtman, & M.I. Damaj, Ph.D. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Nicotine is the main addictive component of tobacco that plays a major role in dependence. Emerging evidence suggest that the endogenous endocannabinoid system may modulate these effects. 2-AG is the most abundant endocannabinoid in the brain, required for retrograde transmission and exerts its action via CB1 receptors. We hypothesize that 2-AG neurotransmission is altered during Nicotine Dependence (ND). We first evaluated 2-AG levels in relevant brain regions after acute administration of nicotine (1, 2mg/kg s.c.) using liquid chromatography-mass spectrometric (LC-MS) method. Next, assessed the effect of repeated nicotine injections (2 times a day for 7 days) on 2-AG levels in LC-MS. Unfortunately, in either treatment group we did not see an effect of nicotine on 2-AG neurotransmission. However, this effect could be due to the sensitivity of the assay. Secondly, there could be a dilution of effects since we take whole tissue. We then wanted to measure the effect of enhanced 2-AG via MAGL inhibition by JZL184 in ND. JZL184 is a potent and selective inhibitor of MAGL. Nicotine reward in the mouse was evaluated in an unbiased conditioned place preference paradigm (CPP) in induction. Our results showed that degradation of MAGL dose-dependently decreased nicotine preference compared to nicotine control in our CPP paradigm. Finally, we assessed JZL184's effect on nicotine

withdrawal. Our results verified that JZL184 dose-dependently decreased somatic signs. Our results suggest that MAGL inhibition may be a therapeutic target in ND. P50DA005274

DETERMINING RAI1 BINDING SITES WITHIN *CLOCK*. C.A. Bax<sup>1</sup>, S. Williams<sup>2</sup>, S. Elsea<sup>2</sup>, & D.L. Zies<sup>1</sup>, Department of Biological Sciences, <sup>1</sup>University of Mary Washington, Fredericksburg, VA 22401, <sup>2</sup>Virginia Commonwealth University, Richmond, VA 23284. Smith-Magenis Syndrome (SMS) is a neurobehavioral disorder characterized by mental retardation, sleep disturbances, obesity and self injurious / attention seeking behavior. The major symptoms of SMS are caused by haploinsufficiency of the retinoic acid-induced 1 (*RAI1*) gene, which is thought to be a transcription factor involved in the regulation of many other genes. A Chromatin Immunoprecipitation-microarray chip (ChIP-chip) experiment was conducted to determine genes regulated by RAI1. One of the genes identified was Circadian Locomotor Output Cycles Kaput (*CLOCK*). The purpose of this project was to confirm RAI1 as a regulator of *CLOCK* and identify specific binding sites within the gene. Transient transfection and luciferase assays were performed. A statistically significant increase in transcription of luciferase was seen when RAI1 was cotransfected with the regulatory region of *CLOCK*. No significant drop in luciferase levels was seen when the shortened *CLOCK* construct was cotransfected with RAI1. Therefore, the binding site is located within the 227 bases that were not removed by the deletion. The identification of a specific RAI1 binding site would enable researchers to identify other genes regulated by RAI1. Knowing the cohort of genes regulated by RAI1 would provide a better understanding of the specific function of *RAI1* and the downstream effects that lead to the symptoms of SMS.

BOTH POLYCYSTIC OVARY SYNDROME (PCOS) AND NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) ARE DISEASES OF METABOLIC SYNDROME SPECTRUM. T.P. Tran<sup>1,2</sup>, A. Baranova<sup>1,2</sup>, A. Biredinc<sup>1,2</sup> & Z.M. Younossi<sup>2,3</sup>, <sup>1</sup>Betty and Guy Betty Center for Integrate Research, Iona Health System, Falls Church VA 22042, <sup>2</sup>Center for the Study of Genomics in Liver Diseases, Molecular and Microbiology Department, George Mason University, Fairfax VA 22030, <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church VA 22042. Obesity has been widely known to cause non-alcoholic fatty liver disease (NAFLD) and aggravate polycystic ovary syndrome (PCOS). Insulin resistance (IR), a hallmark of metabolic syndrome is observed in 50–80% of women with PCOS and patients with NAFLD. Here, we performed a systematic review using PubMed-search for peer-reviewed articles related to PCOS and NAFLD. Based on the association of PCOS and other metabolic abnormalities, such as IR, hyperandrogenism, obesity and NAFLD, the candidate genes have been proposed for PCOS. Closer scrutiny of these genes places most of their proteins at the crossroads of three highly inter-related conditions: metabolic syndrome, obesity and NAFLD. We showed that the prevalence of both PCOS and NAFLD rises proportionally to the degree of IR and increases in the mass of adipose tissue. These conditions often co-exist and may respond to similar therapeutic strategies. We suggest that it is appropriate to consider PCOS as the ovarian manifestation of

metabolic syndrome, similarly to NAFLD that was earlier proposed as the hepatic manifestation of metabolic syndrome.

**THE ROLE OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN MORPHINE DEPENDENCE.** Lindsay M. Kondo & M. Imad Damaj, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. Opiate addiction is a growing concern in today's society. Opiate users show little success in maintenance treatments and improved clinical therapies are needed. Evidence suggests that neuronal nicotinic acetylcholine receptors (nAChRs), which contribute to nicotine dependence, may also influence morphine dependence. We used two complementary approaches, genetically modified mice and pharmacological ligands, to study the involvement of  $\alpha 6^*$  nAChRs (\* denotes additional subunits). In the first experiment,  $\alpha 6^*$  knockout (KO) mice and wild type (WT) littermates were injected twice a day with ascending doses of morphine (20, 40, 80, 100 mg/kg morphine, s.c.) for 8 days. On day 9, mice received a pretreatment of morphine two hours before naloxone challenge (2 mg/kg, s.c.) and abstinence signs were observed for 30 minutes including jumps, paw tremors, head shakes, ptosis, backing, and diarrhea. We observed that the KO mice showed an increased intensity of withdrawal signs in comparison to the WT controls. To complement our transgenic approach, we used a peptide-like  $\alpha 6\beta 2^*$  selective antagonist,  $\alpha$ -conotoxin MII-[H9A;L15A]. In this study, mice were injected three times per day for three days with ascending doses of morphine (50, 75 and 100 mg/kg morphine, s.c.). On day 4, mice received a morphine pretreatment followed by an injection of  $\alpha$ -conotoxin MII-[H9A;L15A] (12 pmol, i.c.v.) 5 minutes before naloxone. MII-[H9A;L15A] treatment enhanced the intensity of global somatic withdrawal signs ( $p < 0.05$ ). Taken together, these results suggest that  $\alpha 6^*$  nAChRs offer a novel target for the treatment of physical morphine dependence.

**AUTOLOGOUS PLATELET-RICH PLASMA (PLATELET GEL) ENHANCES BLOOD FLOW IN LARGE SOFT TISSUE WOUNDS IN VIVO AND REDUCES BACTERIAL GROWTH IN VITRO.** B.F. Host, F. Li & B. Hargrave, Center for Bioelectronics, Old Dominion University, Norfolk VA 23508. Platelet-rich plasma (PrP) enhances the process of wound healing in soft tissues. Platelets in whole blood were concentrated 7-10 times using a Harvest Technologies Concentrator. Concentrated platelets were then placed into a sterile pulsing cuvette and activated by exposure to a 30-kV/cm electric field for 5 pulses, with each pulse having a duration of 300 ns. With the animal under a surgical plane of anesthesia, a 3x8 cm surgical wound was created on the dorsal surface of each animal, and the autologous platelet-rich plasma (formed from rabbit blood) was applied directly to the wound site. To assess the return of blood flow to the wounded area, the wound was imaged using a Moor Instruments Laser Doppler Imager just prior to the creation of the wound, and also post-operatively on days 0, 3, 7, 14, and 21. The wounds of 4 animals were treated with saline and these animals served as our controls. The wounds of 6 animals were treated with PrP. Results are reported as the mean  $\pm$  standard deviation. Our current data suggest that the return of blood flow to the distal region of the wound was significantly greater in the PrP-treated animals than in the control animals treated with saline.

IDENTIFICATION OF GENES THAT MEDIATE ETHANOL-INDUCED ACUTE FUNCTIONAL TOLERANCE IN *C. ELEGANS*. Ka-Po Leung, M. Bolling, J. Gardner, A. Davies & J. Bettinger, Department of Pharmacology and Toxicology, VCU, Richmond, VA 23298. Alcohol abuse and alcoholism are prevalent diseases in our society. There are few adequate treatments available, in part because the molecular mechanisms behind the development of alcoholism are still unclear. We know from human genetics studies that there is a significant genetic component that influences disease susceptibility, and that an individual's development of acute functional tolerance (AFT) after alcohol consumption are strong predictors of lifetime development of addiction. We have taken a genetic approach to study the development of tolerance to ethanol. We used *C. elegans* as a behavioral model for the study of alcohol responses because of its simple nervous system and conserved neurobiology. We performed a genetic screen looking for mutations in genes that are required for the development of AFT to ethanol. Previous studies have shown that the gene NPR-1, a G protein-coupled neuropeptide receptor in *C. elegans* that is homologous to the mammalian neuropeptide Y (NPY) receptor, negatively regulates the development of AFT; animals that lack NPR-1 function develop tolerance to ethanol at a faster rate relative to animals with intact NPR-1. We used an inactivated *npr-1* gene strain, *npr-1(0)*, as a sensitized background for a phenotypic screen to identify animals that are unable to develop AFT. We have identified a mutation, *bet11*, that causes mutant animals to be defective in the development of AFT. Identifying the genes that are responsible for alcohol-induced development of AFT will provide a better understanding of the mechanisms that cause alcohol abuse and alcoholism.

THE GALACTOLIPID SULFATIDE IS A NEGATIVE REGULATOR OF CELL PROLIFERATION. S.A. Freeman, A.D. Pomier & J.L. Dupree, Department of Anatomy and Neurobiology, Virginia Commonwealth University, School of Medicine, Richmond VA, 23284. Myelin is an electrically insulating sheath that oligodendrocytes wrap around neurons, greatly increasing the speed and efficiency of electrical signaling between neurons. Unlike other membranes, myelin is lipid rich and composed of approximately 70% lipid. Myelin is also unique in that it contains an abundance of galactolipids. The two most prominent myelin galactolipids are galactocerebroside and its sulfated derivative sulfatide, made by the enzyme cerebroside sulfotransferase (CST). The CST null mouse exhibits an increased population of oligodendrocytes due to enhanced proliferation and decreased cell death in the spinal cord, but the mechanism of these changes is not known. The goal of this project is to determine if the inhibition of sulfatide results in increased proliferation in Madin Darby canine kidney (MDCK) cells, a sulfatide expressing, immortal cell line. In separate experiments, we will utilize sodium chlorate to block sulfatide production. The sulfatide antibody O4 and bromodeoxyuridine (BrdU) will be used to examine the amounts of sulfatide and proliferation, respectively. Preliminary results suggest that MDCK cells treated with sodium chlorate exhibit an elevated cell population via a mechanism favoring increased proliferation, and additionally, sodium chlorate treated cells show decreased morphologic complexity, which is consistent with findings in the CST null mouse. Understanding the complex

factors that regulate oligodendrocyte proliferation is critical for future production of therapeutics for multiple sclerosis patients.

THE RELATIONSHIP BETWEEN PHYSICIAN SPECIALIZATION AND COMMUNICATION EFFICACY. Amber Collier<sup>1</sup>, Matthew Cronin<sup>1</sup>, <sup>1</sup>School of Management, George Mason University, Fairfax, VA 22030. A central challenge in being an effective physician is communication; patients need reassurance and emotional support, and also need to be informed about risks and requirements of treatment. The challenge is for the physician to be compassionate and communicate complex information in a way that the patient can understand. As physicians become more specialized, these functions may become more difficult to perform. This presents a challenge as the patients who go see specialists often do so because their health conditions are more serious. If specialists are having greater difficulty with communication this could have significant effects on the patient's health outcome. Currently, there is no existing research that explores this area of communication and its corresponding relationships. Thus, this project aims to discover whether and how increased specialization negatively affects the quality of communication, and ultimately the patient's health. We plan to achieve this through survey scales that gauge different dimensions of communication and objective/subject health measures. If we find it does, we hope to be able to suggest mitigation measures.

COMPONENTS OF THE METABOLIC SYNDROME AND THEIR RELATION TO NAFLD IN MORBIDLY OBESE PATIENTS. C.T. Nguvenngo<sup>1</sup>, A. Baranova<sup>2,3</sup>, Z. Younossi<sup>3</sup>, M.J. Estep<sup>1,3</sup>, <sup>1</sup>Biology Department, College of Science, George Mason University, Fairfax, VA 22030, <sup>2</sup>School of Systems Biology, College of Science, George Mason University, Fairfax, VA 22030, and <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA 22042. Non-alcoholic fatty liver disease (NAFLD) and its most severe form non-alcoholic steatohepatitis (NASH) are often referred to as the hepatic manifestations of metabolic syndrome. However, firm connections between individual components of metabolic syndrome and the progression of NAFLD have yet to be conclusively established. The aim of this study was to examine the relationship between lipid profiles and NAFLD progression in morbidly obese patients. Circulating concentrations of fasting glucose, HDL, LDL, Total cholesterol, and triglycerides was obtained using Cholestech-GLU kits. Descriptive statistics, Mann-Whitney U-tests, and Spearman correlations were calculated. Of the 416 NAFLD patients, 245 had progressed to NASH. Significant differences were seen between NASH and non-NASH NAFLD in circulating concentrations of HDL, Triglycerides, and Glucose. Diagnosis of NASH and circulating concentrations of HDL were negatively correlated, while NASH and Triglyceride levels and NASH and glucose levels were positively correlated. The individual components of metabolic syndrome are associated with stage of NAFLD progression to NASH.

LIVER FUNCTION IN HCV PATIENTS WITH DIFFERENT IL28B ALLELIC GROUPS. R. Guragain<sup>1</sup>, A. Baranova<sup>2,3</sup>, Z. Younossi<sup>3</sup>, & M. Estep<sup>1,3</sup>, <sup>1</sup>Bio. Dept., <sup>2</sup>School of Sys. Bio., George Mason University, Fairfax VA, and <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church VA. IL28B genotype is an important

host factor in treatment of Hepatitis C virus (HCV) infection. HCV patients could be either homozygous for “C” allele (CC-group), or homozygous for “T”/ heterozygous (non-CC-group). The success rate of achieving Sustained Viral response (SVR) in the CC-group is significantly higher. However, other host-related factors that affect liver function may also affect rates of SVR achievement. We examined distinctions in clinical parameters between CC-group and non-CC-group HCV patients. Blood samples and laboratory data were obtained for 56 patients who had undergone standard HCV treatment. DNA was extracted using QIAmp Minikit (Qiagen). Allelic discrimination was performed by tetra-primer PCR and electrophoresis. Spearman correlations and Mann-Whitney tests were performed. Of the 23 patients that comprised the CC group, 15 had achieved SVR. Of the 33 non-CC-group patients, only 8 had achieved SVR. SVR negatively correlated with occurrence of significant steatosis ( $r = -0.31$ ,  $P = 0.03$ ). Interestingly, among the patients that achieved SVR, those with CC genotype had significantly increased liver enzymes compared to non-CC-group (average ALT levels 59.75U/l and 35.4U/l, respectively ( $P = 0.007$ )). Similar trends were observed for AST levels (86.91U/l vs. 63.8U/l ( $P = 0.02$ )). Additionally, urine nitrogen was also significantly higher in the CC-group that achieved SVR (18.5mg/dl vs. 8mg/dl, ( $P < 0.001$ )). The results support the hypothesis that factors affecting liver function such as steatosis may have a more significant impact on the achievement of SVR in the non-CC-group.

HOMA CALCULATOR TO CALCULATE RESISTANCE SCORE USING HOMEOSTASIS MODEL ASSESSMENT. L. Alomair<sup>1</sup>, L. Wang<sup>1</sup>, A. Biredinc<sup>1,2</sup>, J.M. Estep<sup>2,3</sup> & A. Baranova<sup>1,2,3</sup>, <sup>1</sup>School of Systems Biology, College of Science, George Mason University, Fairfax, VA, USA, <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church, VA, USA, <sup>3</sup>Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA, USA. Insulin is a protein that plays a major role in glucose metabolism. Insulin may be quantified in the serum as whole insulin or as C-peptide, where C-peptide is more stable to degradation in during sample processing and storage. C-peptide is a byproduct created when the hormone insulin is produced. Equimolar amounts of C-peptide and insulin are then stored in secretory granules of the pancreatic beta cells and both are eventually released to the portal circulation. Homeostasis Model Assessment (HOMA) scores are a combination of fasting insulin and glucose measurements in the serum. HOMA indices are used to assess insulin resistance (IR). Currently, there no publically available software to calculate HOMA scores using C-peptide data. Here we present a software tool capable of calculation of Homeostasis Model Assessment (HOMA) scores based on both fasting C-peptide and fasting insulin levels. Use of this tool will allow researchers to optimize their analysis of insulin resistance in human subjects.

THE DISCRIMINATIVE STIMULUS EFFECTS OF NITROUS OXIDE IN MICE. Kellianne J. Richardson & Keith L. Shelton. Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Despite the high prevalence of clinical and illicit use, the *in vivo* actions of nitrous oxide are unclear. *In vitro* studies have shown that nitrous oxide alters the function of NMDA, GABA<sub>A</sub>, and nicotinic acetylcholine receptors, among others. However, the receptor

system or systems responsible for the intoxicating, subjective effects of nitrous oxide are unknown. Our objective was to use drug discrimination in mice to assess the neurotransmitter systems responsible for producing the subjective stimulus effects of nitrous oxide. Sixteen male B6SJLF1/J mice are being trained to discriminate 10 min of exposure to 60% inhaled nitrous oxide from 100% oxygen. Discrimination training under conditions in which training and test sessions were done in room air after cessation of nitrous oxide exposure resulted in lengthy training requirements and poor stimulus control. As a result the procedure was modified to conduct discrimination training and testing while being concurrently exposed to nitrous oxide. Thirteen mice thus far have acquired the discrimination and begun substitution testing with increasing nitrous oxide concentrations. The results indicate that under proper training conditions nitrous oxide can serve as a discriminative stimulus. Further testing will be conducted using site-selective test drugs to determine the neurotransmitter systems responsible for the discriminative stimulus effects of nitrous oxide.

CHARACTERIZATION AND OPTIMIZATION OF THE SYNAPTONEUROsome PREPARATION IN ORDER TO INVESTIGATE ETHANOL INDUCED ALTERATIONS TO THE SYNAPTIC TRANSCRIPTOME. Megan A. O'Brien & Michael F. Miles, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA 23298. It is well established that mRNA can be transported to neuronal distal processes, where it can undergo localized translation regulated in a spatially restricted manner in response to stimulation, potentially playing a role in synaptic plasticity that results in long-term adaptive brain responses. In order to investigate our hypothesis that ethanol behavioral sensitization results, at least in part, from alterations in the trafficking of mRNAs to distal processes, we have worked to optimize a protocol for obtaining synaptoneurosomes from the frontal pole of mice treated with repeated ethanol. In the protocol, fresh tissue undergoes homogenization and fractionation resulting in a pelleted fraction (P2) that should be enriched with vesicularized pre- and post-synaptic elements from which RNA and protein can be extracted. Characterization of the preparation through electron microscopy, western blotting, and quantitative PCR indicate synaptic enrichment of the putative synaptoneurosomes fraction. Attempts to further isolate synaptoneurosomes were undertaken through an immunoaffinity purification scheme targeting the synaptic transmembrane protein, Neuroligin. We conclude that the synaptoneurosomes preparation will provide us with samples enriched in synaptically localized mRNAs and proteins that will aid our investigation into the underlying molecular alterations that contribute to behavioral sensitization in response to repeated ethanol.

CHRONIC INHIBITION OF MONOACYLGLYCEROL LIPASE REDUCES THE INTENSITY OF PRECIPITATED WITHDRAWAL SIGNS IN OPIOID DEPENDENT MICE. R.A. Owens<sup>1</sup>, D. Ramesh<sup>1</sup>, B.F. Cravatt<sup>2</sup> & A.H. Lichtman<sup>1</sup>, <sup>1</sup>Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, 23298. <sup>2</sup>Scripps Research Institute, La Jolla, California, 92037. Chronic use of opioids can lead to dependence that results in a clinically significant withdrawal syndrome upon drug cessation. It has been shown that

$\Delta^9$ -tetrahydrocannabinol (THC) reduces opioid withdrawal signs in rodents via CB<sub>1</sub> receptors. The endocannabinoids, anandamide (AEA) and 2-arachidonylglycerol (2-AG), also activate CB<sub>1</sub> receptors, but they are rapidly metabolized by their respective enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Research from our laboratory suggests that acute administration of these catabolic enzyme inhibitors reduces naloxone-precipitated withdrawal signs in morphine-dependent mice. In the present study, we investigated whether the MAGL inhibitor JZL184 would retain its anti-withdrawal effects following repeated administration. ICR mice were implanted with 75 mg morphine pellets, and challenged 72 h later with the opioid receptor antagonist naloxone to precipitate withdrawal. The presence of somatic withdrawal signs such as jumps, front paw flutters, weight loss, and the occurrence of diarrhea were measured. As previously reported, acute treatment of THC (10mg/kg) significantly reduced the occurrence of jumps and diarrhea. Repeated treatment with high dose of JZL184 (40 mg/kg), but not the low dose (4 mg/kg), reduced all measured signs of opioid withdrawal. The results of the present study indicate that inhibitors of MAGL offer a promising target to treat opioid dependence.

LYSOPHOSPHATIDIC ACID UPREGULATES EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA IN OVARIAN CANCER CELLS. J.K. Ngwainmbi, A. Mukherjee & F. Fang, Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. Ovarian cancer is the fifth most common cancer among women in the United States and it is the leading cause of death among all gynecological cancers. Most early stage ovarian cancers are detected incidentally on routine pelvic examinations, and five year survival rates of over 90% can be achieved for localized disease. Lysophosphatidic acid (LPA), is a soluble phospholipid made up of a single fatty acyl chain, a glycerol back bone and a free phosphate group. It stimulates proliferation, survival, migration and invasion of ovarian tumor cells by acting on its cognate G protein-coupled receptors. Previous studies by others suggest that LPA binds and activates *peroxisome proliferator-activated receptor gamma* (PPAR $\gamma$ ), a nuclear transcription factor important in regulation of fat and energy metabolism. In the current study, we did not observe any evidence for direct interactions between LPA and PPAR $\gamma$  instead, we demonstrated that LPA induces the expression of PPAR $\gamma$  in ovarian cancer cells leading to an increase in its transcriptional activity. This work points to a novel mechanism for activation of PPAR $\gamma$  by LPA in mammalian cells.

### Natural History & Biodiversity

TWELVE YEARS LATER: ECOLOGICAL COMMUNITIES OF THE ABRAMS CREEK WETLANDS IN WINCHESTER AND FREDERICK COUNTY, VIRGINIA. Briana L. Barron, James E. Bisset, Linden E. Lewis, Daniel A. Milhon, Cory M. Miller, Benjamin S. Sawyer, Amy L. Smith, John V. Stevens & Woodward S. Bousquet, Environmental Studies Department, Shenandoah University,



Winchester VA 22601. Located in northwestern Virginia, the Abrams Creek Wetlands support 18 vascular plant species and two community types (calcareous muck fen, wet-mesic prairie) considered rare in the state. A 1998 study by Shenandoah University (SU) supplemented investigations by Virginia's Natural Heritage Program in documenting the property's ecological significance. In the spring and fall of 2010, SU researchers resurveyed three communities and studied one new community at this site. Using the relevé (Braun-Blanquet) method, community plots were described by their vegetation and physical characteristics. The new plot includes *Carex lasiocarpa*, which is listed as critically imperiled (S1) in Virginia. Percent similarity comparisons of the three resurveyed plots show substantial change (PS = 15.5%, 17%) in two but little change (93.8%) in the third since 1998. Recommendations for managing the Abrams Creek Wetlands include removing invasive species and minimizing future disturbances from the area's planned housing developments and walking-biking paths.

REPRODUCTIVE BEHAVIOR OF THE POTOMAC SCULPIN (*COTTUS GIRARDI*). Kimberly J. Bolyard, Dept. of Biology, Bridgewater College, Bridgewater, VA 22812. Potomac sculpin (*Cottus girardi*) fish from Dry River, Virginia were brought into the laboratory to study their reproductive behavior and to evaluate if these fish are subject to sexual selection pressures. Reproductively mature males vary in standard length. I tested the hypothesis that females should prefer to mate with larger males because they should be able to defend the nest and eggs from predators better than smaller males. Studies were conducted during the breeding season, February-March, 2010 and 2011. Observations were made of groups consisting of two males, one large and one small, and one female. Male reproductive behavior included pectoral fin waving and body shaking associated with darkening the body color. An actively courting male would approach the female and bite her in the head region while turning her towards his nest cavity. If she entered the nest, the male would rest on her while shaking intermittently. Occasionally, the male would leave the female to chase the other male away from the nest area. More females, six of seven, associated with or spawned with the larger of the two males. While not statistically significant, the observations suggest that female mate choice may play a role in the reproductive ecology of these fish. In addition, Potomac sculpins may be subject to male-male competition for nest sites and for females; hence, intrasexual selection will be the focus of future studies.

INVESTIGATION OF PRE- AND POST-ZYGOTIC REPRODUCTIVE BARRIERS BETWEEN TWO HOST-COMPLEX RACES OF A PARASITIC WASP. Justin P. Bredlau & Karen M. Kester, Dept. of Biology, Virginia Commonwealth University, Richmond VA 23284. Understanding the mechanisms of speciation and reproductive isolation has important implications for studies in biodiversity, evolution, and ecology. Insect parasitoids in particular display rapid speciation that may be associated with chemically diverse host plants. Investigations of host-associated differentiation of parasitoids have largely focused on the degree of molecular genetic differentiation, but a true test of species status must consider whether differentiated populations are capable of interbreeding and producing viable offspring. We examined possible mechanisms of isolation between two genetically

distinct (2% divergence in the mtDNA COI locus) host-plant complex races of the parasitoid, *Cotesia congregata* (Hymenoptera: Braconidae) originating from *Manduca sexta* on tobacco and *Ceratomia catalpae* on catalpa. We compared male responses to female pheromones, elements of male acoustic courtship signals, and mating success between the two groups. Males responded to female pheromones of either race and male courtship songs showed only subtle differences, suggesting that factors other than courtship behavior may be involved in pre-zygotic isolation of the two races. Wasps from the two sources mated and produced offspring; however, 90% of females from one hybrid cross failed to produce offspring, leading to post-zygotic isolation. Development time, emerged brood size, and sex ratios among the wasp races also differed. Results suggest that the two host-complex races represent distinct sibling species.

MOLECULAR AND MORPHOLOGICAL VARIATION IN THE BARNACLE PREDATOR *NEMERTOPSIS BIVITATTA* (NEMERTEA, HOPLONEMERTEA). Serena A. Caplins<sup>1</sup>, Jon. L. Norenburg<sup>2</sup>, & Clint Turbeville<sup>1</sup>, <sup>1</sup>Dept. of Biol., Virginia Commonwealth University, Richmond Va. 23284, <sup>2</sup>National Museum of Natural History, Smithsonian Institution, Washington D.C. The nemertean worm *Nemertopsis bivitatta*, is a member of the hard-bottom intertidal community, occurring along the coasts of Europe, Brazil, and the Southeastern United States. Our laboratory observations indicate that this nemertean is a suctorial feeder of barnacle crustaceans. Individuals of *N. bivitatta* are typically pale yellow to whitish in color with a pair of dorsally-situated dark greenish brown pigment bands that extend nearly the entire length of the worm. In most individuals these stripes are separated anteriorly, but in some individuals they are fused anteriorly. Variation in stripe configuration has been considered to reflect intraspecific variation. We describe variation in stylet basis morphology and assess genetic variation, which may be correlated with stripe configuration. Sequences of the mitochondrial cytochrome-oxidase one gene (CO1, 500-650 bps) were obtained for 8 individuals exhibiting the anterior fusion of pigment bands and for 14 individuals without this feature. A pair-wise comparison revealed a 14.8% difference exists between the morphs. This lies within the range of variation observed between species in Hoplonemertea. A tree-based approach will be used to further test whether these morphs represent different species.

TADPOLE DENSITY CHANGES THE RELATIONSHIP OF RED-EYED TREEFROG MORPHOLOGY AND JUMPING PERFORMANCE. J.F. Charbonnier<sup>1</sup>, T. Landberg<sup>2</sup> & J. Vonesh<sup>1</sup>, <sup>1</sup>Department of Biology, Virginia Commonwealth University, Richmond VA 23298 and <sup>2</sup>Department of Biology, Boston University, Boston MA 02215. As organisms develop, increased body size is often accompanied by shape changes that alter the morphology-performance relationship. Animals with different growth histories may also have different shapes at similar body sizes. To investigate how larval growth history affects the morphology-performance relationship, we raised red-eyed treefrog tadpoles (*Agalychnis callidryas*) at three densities (5, 25 and 50 tadpoles per 400 L tank) and measured jump distance during metamorphosis. We predicted that tadpoles grown at low density would metamorphose into larger frogs with relatively longer legs than

those grown at higher densities. We also expected low density frogs to jump further – both absolutely, because of their larger body size and relative to their size if they had longer legs. Frogs from low density had longer snout-vent lengths (SVL) than those from medium and high densities and longer tibiafibula lengths and greater masses relative to their SVL. Jump distance was strongly correlated with tibiafibula length; however, there was a significant density\*tibiafibula interaction. While longer tibiafibulas in high and medium density frogs were correlated with longer jump distances, there was no such correlation in the frogs from low density tanks. We interpret these results as a consequence of the greater relative mass of the frogs from low density tanks. The relatively long legs of these frogs may not fully compensate for their disproportionately higher mass. This study demonstrates that different larval densities can change not only red-eyed treefrog morphology, but also the morphology–performance relationship. It suggests a trade-off where low larval density can increase body size and presumably fat reserves, but the latter may decrease jumping performance.

FLEAS (SIPHONAPTERA) FROM A SMALL MAMMAL COMMUNITY IN A GUATEMALAN CLOUD FOREST. R. P. Eckerlin<sup>1</sup>, W. Bulmer<sup>1</sup>, H. Lanier<sup>2</sup>, J. O. Matson<sup>3</sup> & N. Woodman<sup>4</sup>, <sup>1</sup>Natural Sciences Div., Northern Virginia Community College, Annandale, VA 22003, <sup>2</sup>Dept. Biological Science, University of Alaska-Fairbanks, AK 99775, <sup>3</sup>Dept. of Biological Sciences, San Jose State University, San Jose, CA, 95192, <sup>4</sup>USGS Patuxent Wildlife Research Center, U.S. National Museum, Smithsonian Institution, P.O.Box 37012, Washington, DC 20013. The flea fauna of a mammal community at a specific site has never been recorded for Guatemala. We collected 104 small mammals (6 spp. shrews & rodents) and 323 fleas from remnant cloud forest at 2950-3160m in El Retiro, Huehuetenango, Guatemala. Fleas were brushed off the hosts, preserved in alcohol and later mounted on slides for identification, *Phsaetis vermiformis*, previously known from a single specimen in Guatemala, was the most common flea but found only on *Peromyscus beatae* and *P. guatemalensis*. An undescribed species of *Ctenophthalmus* was present on *Sorex saussurei*. *Phsaetis mathesoni*, found on several rodent host species, is a new country record for Guatemala. A single specimen of an *Athyloceras* sp. from *P. beatae* extends the range of this genus south of central Mexico. Other species present on rodents were *Ctenophthalmus sanborni*, *Baculomeris schmidt*, *Kohlsia osgoodi*, *Jellisonia painteri*, and *Strepsylla* sp. Most of the 3 species of *Reithrodontomys* that we examined had no fleas. Although the mammal diversity was judged to be low the flea fauna was robust and diverse.

TROPHIC DYNAMICS OF CHESAPEAKE BAY PLANKTON. Todd A. Egerton, Matthew R. Semcheski & Harold G. Marshall, Department of Biological Sciences, Old Dominion University, Norfolk VA 23529. Examining the relationship between diversity and ecosystem processes has become an important topic in ecology given the increased rate of species extinctions. Previous work has shown that stations in lower Chesapeake Bay with increased nutrient concentrations have higher productivity and algal abundances but lower phytoplankton diversity. However, the potential effect of algal diversity on higher trophic levels has not been examined. Using a 16 year dataset (1986-2001) of phytoplankton and zooplankton community

composition, water quality parameters, and commercial fisheries landings, several relationships were identified. Zooplankton diversity is positively correlated with phytoplankton diversity and negatively correlated with total phytoplankton abundance. Long-term trend analysis indicates a significant decline in zooplankton abundances, as well as a significant negative correlation between total nitrogen concentrations and zooplankton species richness. Furthermore, there has been a significant decline in the average annual landing of Virginia commercial fisheries. Fishing yield is positively correlated with zooplankton abundance and negatively correlated with algal productivity. This suggests that bottom up factors are driving the system, where increased nutrient levels lead to more productive less diverse algal communities capable of supporting fewer, less diverse grazers and an overall decline in fish populations. Supported by VADEQ.

EFFECT OF PHOTOPERIOD ON IMMATURE DEVELOPMENT IN THE TIGER MOSQUITO, *AEDES ALBOPICTUS*. Allen Frisa & Deborah Waller, Dept. of Biol., Old Dominion Univ., Norfolk, VA 23529. Tiger mosquitoes are active from spring through fall and overwinter in the egg stage. We examined whether mosquito development varied depending on the photoperiod the immatures experienced. Late-instar larvae were collected from an incubator with a summertime photoperiod (16L:8D), placed in individual cups with 20 ml deionized water and fish food, and incubated in the following photoperiods: constant dark (DD), spring/fall (12L:12D), summer (16L:8D), reverse summer (8D:16L) and constant light (LL). There were ten replicates per photoperiod. Time from pupal formation to adult eclosion was recorded along with the gender of the adults. Under constant temperatures with a mean of 26°C, pupal durations were 2-3 days for both male and female with no differences related to photoperiod. In a second experiment in which spring/fall and constant light incubators were kept at a mean of 23°C, pupal durations were 3-4 days for both males and females. Therefore temperature influenced developmental rates but there was no influence of photoperiod on pupal duration.

A SURVEY OF MUSKRAT INTESTINAL PARASITES IN VIRGINIA. Jeronimo G. Da Silva Neto, Elizabeth R. Hepner, Allison E. Salinger, Emily A. Kimminau & Lisa K. Belden, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. Infectious diseases of humans and wildlife have increased dramatically in recent decades. These diseases can have important impacts on global health, agriculture and biodiversity. Despite these increases, we still know relatively little about disease in natural wildlife populations. In many cases, we lack even basic information about what parasites and pathogens are present in wildlife species. This prompted us to conduct a survey on local populations of muskrats, a common semi-aquatic rodent found in the United States. We conducted necropsies on donated muskrat carcasses collected from across Virginia, and began to establish a baseline database on parasites of muskrats, with a focus on the macroparasitic worms in the gastrointestinal tract (stomach, small intestine, large intestine and cecum). Since 2008, we have necropsied over 100 individuals. Three genera of parasitic flatworms (trematodes) dominated the samples – *Echinostoma*, *Quinqueserialis* and *Wardius*. As these parasites have complex life cycles involving multiple hosts in addition to muskrats, we can potentially use the presence of these parasites to infer

something about the ecological communities of the sites at which the muskrats were collected. We have also completed some analyses regarding the distribution of the parasites among hosts. We have not seen any significant relationship between total worm burden and either sex or mass of the host. Our research provides an important baseline for understanding parasite dynamics in natural muskrat populations. This allows a better understanding of potentially emerging diseases and can improve how we manage wildlife disease.

ALLEGHENY WOODRATS (*NEOTOMA MAGISTER*) IN VIRGINIA: COMPARISONS OF OCCUPANCY OVER TIME. Jennifer M. Kanine, Michael T. Mengak & Steven B. Castleberry, Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia 30602. The Allegheny woodrat (*Neotoma magister*) is a cryptic small mammal that occupies rocky outcrop and cave habitats throughout the Appalachian Mountains. Populations have been documented as declining throughout much of its historic range. Population declines have been attributed to a suite of effects occurring in the habitat including interactions with raccoon roundworms, loss of American chestnut, habitat reduction, and fragmentation. We surveyed 232 sites in Virginia in 2008-10 for woodrat occupancy and compared our results to results of surveys conducted from 1990-1999 to assess changes in woodrat occupancy over time. We also examined if our trapping protocol was adequate to determine occupancy through a combination of extending the number of nights trapped and through the use of remote cameras. Naïve occupancy during the 1990s was 67%, while only 49% of sites were occupied from 2000-2010. Woodrat populations exist as metapopulations, where sites consist of spatially separated populations that interact. Metapopulations systems have some groups that may be extirpated in some years which are followed by re-colonization in subsequent years. We have documented 38% of our sites having variable occupancy over time. Results of our camera surveys indicate that our current monitoring protocol is effective at determining occupancy 85% of the time.

A SKULL OF THE EXTINCT RIGHT WHALE *BALAENULA* FROM THE LATE PLIOCENE OF NORTH CAROLINA. Laura Kellam, Alton C. Dooley, Jr., & Vince Schneider. Roanoke College, Salem, VA 24153, Virginia Museum of Natural History, Martinsville, VA 24112, North Carolina Museum of Natural Science, Raleigh, NC 27601. A nearly complete balaenid skull was collected in May 2008 from the Bear Bluff Formation in Lake Waccamaw, North Carolina. The cranium as preserved is 165 cm in length. The tight closure of most of the cranial sutures suggests that this was a mature animal at the time of death. It has extreme telescoping, arched rostral bones, dorsoventrally compressed tympanic bullae, and the dentary has an anterior torsion and lacks a distinct coronoid process, all features that are typical of balaenids. Features shared with the genus *Balaenula* include: 1) small overall size; 2) large, block-like nasals; 3) approximately right angle between posterior and anterior processes of the petrosal; 4) anteriorly oriented squamosal; 5) right angle formed between supraorbital processes of frontals when viewed anteriorly. However, it differs from *B. astensis* from Italy and *B. balaenopsis* from Belgium in a number of characters. This is the first report of *Balaenula* from the Bear Bluff Formation, which has an approximate age of 2.75 Ma. All other reported specimens of *Balaenula* from

the western Atlantic are from the lower Yorktown Formation, with an approximate age of 4.5 Ma. *Balaemula balaenopsis* from Belgium and undescribed *Balaemula* specimens from Japan and California are Pliocene in age, but their ages are poorly constrained, while *B. astensis* from Italy has an approximate age of 3.5 Ma. The Lake Waccamaw specimen is thus possibly the youngest known occurrence of *Balaemula*.

**PRIMARY FEATHER PIGMENTATION IS CONSTRAINED BY FUNCTIONAL NECESSITY.** Joshua D. Kreitzer & Lisa Horth Dept. of Biology, Old Dominion University, Norfolk, VA 23529. Primary feathers are critical for powered flight. High exposure of these feathers to the wind and associated abrasive particles necessitates that they be strong relative to other, less exposed feathers. The black pigment, melanin, has been associated with increased feather strength via an increase in keratin thickness near the melanin and via the added strength provided by the melanin granules themselves. Furthermore, melanin provides protection from the damaging affects of ultraviolet radiation in comparison to paler feathers. We tested the hypothesis that primary feathers would be more frequently pigmented with melanin than a flight-neutral feather patch, the upper back. A survey of 4006 species of non-passerine and 1823 species of passerine birds showed that 63.4% had black in the primaries, 35.4% had other color as the darkest color, and 1.2% had wholly white primaries. The upper back feathers showed a different pattern, with 29.0% of species having black, 69.2% having other, and 2.0% having white. Chi-square analysis showed that the pigmentation of the primary feathers was significantly different from the expected color distribution of the more flight-neutral upper back feathers ( $p$ -value <0.0001). These results may be understood as evidence supporting the hypothesis that primaries are more constrained to be black than the upper back feathers.

**RESPONSE OF IMMATURE MOSQUITOES TO PREDATOR PRESENCE.** Jackie McKee & Deborah Waller, Dept. of Biol., Old Dominion Univ., Norfolk, VA 23529. Mosquito immatures are consumed by a variety of aquatic predators. We tested responses of mosquito larvae and pupae to the presence of dragonfly nymphs, backswimmers and whirligig beetles in the laboratory. Experimental units consisted of 14 cm diam petri dishes with a grid of 2 cm diam squares etched on the bottom. Petri dishes were filled with 80 ml deionized water and set in sleeves that were either black, white or half black/half white. Previous experiments demonstrated that first instar larvae prefer white backgrounds, later instars show no preference, and pupae prefer black backgrounds, and mosquito activity declines with age. A predator was placed in the petri dish and then either a larva or pupa was introduced to the center of the dish. Positions of both the mosquito and the predator were noted every 30 seconds for five minutes. Five different mosquitoes were tested in each experiment. Predators were replaced with a new predator of the same species if it consumed the mosquito. All three predator species ate mosquito larvae and pupae on occasion. There was no response by mosquito immatures to predator presence. Larvae were more active than pupae and pupae moved less frequently on black than on white backgrounds. These patterns are similar to mosquito behavior in the absence of predators, where larvae move frequently to find food and the non-feeding pupal stage seeks out dark areas.

HIGH SPEED EXAMINATION OF PECTORAL STRIDULATION SOUND GENERATION IN BLUE CATFISH *ICTALURUS FURCATUS*. Y. J. Mohajer, M. L. Fine & Z. N. Ghahramani, Department of Biology, Virginia Commonwealth University, Richmond VA 23284-2012. The blue catfish *Ictalurus furcatus* is an invasive species whose population numbers have exploded in Chesapeake Bay estuaries. Catfishes produce stridulation sounds by rubbing ridges on the dorsal process of the pectoral spine against a rough surface on the cleithrum to produce a series of pulses during abduction. We studied the mechanism of sound generation by synchronizing audio recordings with a high speed camera at 2000 frames per second. Unlike channel catfish that typically keep their pectoral fins retracted (adducted), blue catfish tend to maintain them in a forward position. Therefore sound production requires the fish to adduct the spine before forward stridulatory movement. Adduction movements were about a third as long as abductions (means of 49 and 152 ms respectively). The amplitude of abduction and adduction movements was similar at 25.9 and 26.5 degrees. Stridulatory abductions consisted of a series of 3-17 short rapid movements (median of 13) with an average rotation of 3.1 degree over 1.5 ms. Movement was followed by an interpulse period of 13.2 ms in which the spine was stationary. Sound amplitude was low during movement and increased during the stationary period suggesting time was required to activate vibrations in the pectoral girdle. Sounds are produced by a slip-stick mechanism similar to a bow over a violin string.

SYSTEMATIC REVISION OF THE NORTHERN SHORT-TAILED SHREW, *BLARINA BREVICAUDA* (SAY). N. D. Moncrief<sup>1</sup>, Wm. D. Webster<sup>2</sup>, J. R. Choate<sup>3</sup> (deceased) & H. H. Genoways<sup>4</sup>, <sup>1</sup>VA Museum of Natural History, Martinsville, VA 24112, <sup>2</sup>Dept. Biology, Univ. of North Carolina Wilmington, Wilmington, NC, 28403, <sup>3</sup>Sternberg Museum of Natural History, Fort Hays State University, Hays, KS 67601, and <sup>4</sup>University of Nebraska State Museum, Lincoln, NE 68588. The northern short-tailed shrew, *Blarina brevicauda* (Say, 1823) occurs from southern Canada southward to the central Great Plains, in the Appalachian Mountains south to Georgia and Alabama, and along the East Coast as far south as southeastern North Carolina. We analyzed 9 cranial and mandibular measurements of 2,736 specimens using a single classification ANOVA and a principal component analysis. We conclude that *B. brevicauda* consists of 7 well-defined subspecies. Two subspecies (*B. b. brevicauda* and *B. b. talpoides*) occupy almost the entire geographic range of the species. The other 5 subspecies (including 3 that were previously undescribed) occupy small-to-modest geographic ranges at the periphery of the species' range and in isolated geographic areas. We recognize these 7 subspecies because we believe that each has begun to follow its own evolutionary path. Mitochondrial DNA data and our morphological data indicate that most or all of the peripheral subspecies are derived from *B. b. talpoides*. These data also indicate that *B. b. brevicauda* and *B. b. talpoides* are semi-species.

A COMPARISON OF TOOTH BREAKAGE RATES IN MIOCENE CARCHARHINIFORM SHARKS FROM THE CARMEL CHURCH QUARRY, CAROLINE COUNTY, VIRGINIA. Brandi Neifert, Roanoke College, Salem, VA 24153 & Alton C. Dooley, Jr., Virginia Museum of Natural History, Martinsville, VA

24112. Excavation of marine sediments of the middle Miocene Calvert Formation at the Carmel Church Quarry has resulted in the collection of thousands of shark and ray teeth. Unlike most large collections of shark teeth, the Carmel Church specimens were all collected in situ from a single bed with a maximum thickness of less than 1 m. While the majority of teeth from Carmel Church represent clearly reworked specimens, a substantial portion show no evidence of reworking and provide a sample that may represent the local population of sharks over a relatively short period of time. Multiple shark taxa with similar tooth morphologies and body sizes may have coexisted through niche partitioning. This could have been achieved through behavioral variations such as temporal segregation (diurnal or nocturnal habits, or seasonal movements) or through variations in dietary preferences. In order to test for evidence of variation in dietary preferences, tooth breakage frequency and height-thickness ratios were examined in four shark genera that are broadly similar to each other in size and tooth morphology – *Hemipristis*, *Galeocerdo*, *Physogaleus* and *Carcharhinus*. Preliminary results show similar overall breakage rates among the four genera, but a higher rate of catastrophic breakage in *Carcharhinus* and *Galeocerdo*.

MODELING THE PERSISTENCE OF RARE GENOTYPE IN NATURE. Iordanka N. Panayotova, Department of Mathematics and Statistics & Lisa Horth, Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529. Major theoretical constructs explaining the maintenance of rare genotypes include mutation-selection balance and balancing selection. Empirical work demonstrates that mutations arise on the order of  $\sim 10^{-6}$ /gene/generation ( $\pm$  an order of magnitude contingent upon data used). Thus, novel mutants that arise from one or two single nucleotide polymorphisms and that persist at a frequency exceeding the mutation rate (by three or four orders of magnitude) are not trivially explained by mutation-selection balance and are extremely understudied. Empirical demonstrations of negative frequency dependent selection tend to only address the maintenance of polymorphisms that oscillate around a frequency far in excess of  $\sim 10^{-2}$ . Thus, what maintains the rare genotypes that persist at frequencies of  $10^{-2}$ - $10^{-4}$ ? Here, we present a model that incorporates empirical life-history data on mosquitofish from 15 years of field-work. Our model simulates the maintenance of a stable polymorphism where the rare genotype persists at a frequency of  $\sim 10^{-2}$  (the actual frequency of the melanic pigmented genotype in nature). Our results present a novel and intriguing find by providing a robust mechanism for the maintenance of a rare (but not too rare) genotype in a stable polymorphism.

POPULATION GENETICS OF TUNDRA (*CYGNUS COLUMBIANUS COLUMBIANUS*) AND TRUMPETER (*CYGNUS BUCCINATOR*) SWANS: THE IMPACT OF CLIMATE CHANGE ON THE BREAKDOWN OF PRE-ZYGOTIC ISOLATING MECHANISMS. Lauren E Wilson, Department of Environmental Science and Policy, George Mason Univ., Fairfax, VA 220304444. The Tundra (*Cygnus columbianus columbianus*) and Trumpeter (*C. buccinator*) Swans are two migratory swan species with significant breeding populations in Alaska. Historically, breeding ranges have remained sympatric; the Trumpeter nests on boreal forest wetlands while the Tundra nests near tundra wetlands. However,



increased population size of both species and expansion of the boreal forest, a likely result of global climate change, have caused areas of geographic overlap to occur. The Trumpeter and Tundra Swans are known to be reproductively compatible, as a viable hybrid population exists in Virginia at the Airlie Center's Swan Research Program (SRP). Further, accounts from local wildlife managers indicate the presence of fertile hybrids in overlapping breeding areas in Alaska. This research seeks to confirm the presence of viable wild hybrids and to elucidate the genetic structure and relatedness of these interbreeding populations by comparison of phylogenetically relevant genetic loci of wild individuals to known hybrids from the SRP. We will also incorporate "historic" samples collected in 1920 (and prior) to confirm that this interbreeding is a recent phenomenon and likely due to habitat disturbance from climate change.

EFFECTS OF PREDATION, COMPETITION, AND ISOLATION ON THE RATE OF SHELL SELECTION IN THE HERMIT CRAB *PAGURUS LONGICARPUS*. Amber L. King & Deirdre Gonsalves-Jackson, Biology Department, Virginia Wesleyan College. Hermit crabs are most notably known for their use of gastropod shells to protect their soft bodies, which lack calcification on their abdominal exoskeleton. Because of the necessity for a gastropod shell, hermit crabs will often fight amongst each other for shells due to the scarcity of available shells because without a shell to provide adequate protection, they risk exposure. *Pagurus longicarpus* was used in this study to determine the effect of various factors on the rate of shell switching, specifically the effect of isolation, conspecifics, and predation. It was proposed that more hermit crabs would switch shells and the rate of shell switching would increase when isolated, rather than when in the presence of conspecifics or a predator. Three different treatments were set up and based on the results, the two hypotheses were supported because more hermit crabs switched shells and at a faster rate in the isolation treatment, rather than in the other two treatments, suggesting that predation may have posed as significant a threat in my study because the hermit crabs failed to switch shells.

## Psychology

SEX ANXIETY AND SEX GUILT FROM 1980 TO 2010. Yang Deng & Louis H. Janda, Department of Psychology, Old Dominion University, Norfolk VA, 23529. The present study examined perception of social desirability regarding sex anxiety, perceived norms regarding sex guilt, and attitude changes in sex anxiety and sex guilt over the past 30 years. Undergraduate students completed the Sex Anxiety Inventory (SAI) honestly, attempting to present a favorable impression and attempting to present an unfavorable impression. They also completed the sex guilt subscale of Mosher Forced-Choice Guilt Inventory (MFCGI) by describing their own feelings and estimating their same-sex peer responses. Both sex anxiety scores and sex guilt scores were compared among different instructions. The self-descriptive scores on sex anxiety and sex guilt were compared with those in 1979/1980. The results indicated that the favorable-impression scores on sex anxiety were significantly higher than the unfavorable-impression scores and the self-descriptive scores; the

self-descriptive scores on sex guilt were significantly higher than the estimated, same-sex peer scores, consistent with the concept of *pluralistic ignorance*; and the self-descriptive scores on sex guilt in 2010 were significantly higher than those in 1979. The results suggest that people are holding a conservative standard for sex but a permissive perception of sex norm. Changes in sex guilt and sex anxiety between 1979/1980 and 2010 are discussed.

THE EFFECT OF A CONTEXTUAL AID DURING A VISUAL SEARCH TASK. Molly Liechty & Poornima Madhavan, Old Dominion University, Norfolk, VA 23529. Individuals utilize contextual cues (familiar objects within an environment) to simplify their world and anticipate where items will be. The goal of this study was to simulate the way in which airport security screeners utilize contextual cues while searching for threat objects. Twenty undergraduates from Old Dominion University performed a simulated luggage-screening task during which they were connected to an eye tracker. Participants screened 375 bags, 75% of the images contained a target and/or a distractor (an item in the visual scene semantically unrelated to the threat object, i.e., iPod). Results revealed that when the distractor was present, fixation durations increased while fixation counts, dwell times, and saccade counts decreased, demonstrating an aggressive search pattern and an expectation of target presence. We contend that participants formed an implicit association between the distractor and the target and used this association to improve the efficiency of their visual search. This implicit association of objects demonstrates the potential that exists to bias airport security screeners and could aid in training security screeners.

WHEN FEAR AND SADNESS ARE GOOD: THE EFFECTS OF ANGER, FEAR, AND SADNESS ON THREAT RECOGNITION DURING LUGGAGE SCREENING. Ray C. Heikens & Kimberly E. Culley, Dept. of Psychology, Old Dominion University, Norfolk VA, 23529-0001. Research involving the impact of negative emotions on decision-making shows that they can have an effect on one's perception of risk. Anger has a significant reduction in the amount of perceived risk and is able to increase hopefulness for the future. Fear has the opposite effect, increasing perceived risk and reducing optimism. Sadness has a limited history of examination, but can drive one's risk assessment more logically. Current standards of luggage security screening allow personnel to view bags being x-rayed for 4 seconds. The purpose of this study was to investigate the effect of anger, fear, and sadness as well as the effect of stimulus duration on luggage screening accuracy. Participants (n = 228) viewed images selected to induce one of the three test emotions, then attempted to locate threatening objects in x-rays of luggage after either 2, 4, or 6 seconds of viewing. Results showed that 4 seconds of viewing enable the highest percentage of hits when participants were angry or afraid. Sad participants had a higher hit rate when viewing the x-ray for 6 seconds and 6 seconds claimed the lowest percentage of false alarms overall. The implications of this research could aid in improving training and optimizing decision-making for security personnel.

PARENTAL ALCOHOL ABUSE, NEGLECT, PSYCHOLOGICAL ABUSE, AND PHYSICAL ABUSE: A BRIEF LOOK AT INTIMATE PARTNER VIOLENCE. Emily M. Muschek, Old Dominion Univ., Norfolk, VA. Adverse childhood

experiences (parental alcohol abuse, neglect, psychological abuse, and physical abuse) have long-term negative consequences. This study examines the relationship between these 4 types of adverse childhood experiences and emotional and physical abuse in college dating relationships. 426 college students completed 4 measures that assessed their adverse childhood experiences, exposure to parental alcohol abuse, emotional abuse, and physical aggression. Overall, ACOAs were more likely to be in a relationship in which they are being emotionally abused in all four dimensions. ACOA status also predicted restrictive engulfment behaviors as well as behaviors resulting in injury toward their partners. ACOAs were more likely to be in a relationship in which their partner was psychologically aggressive. Participants who were exposed to parental alcohol abuse were more likely to express behaviors that would injure their partner. Participants who experienced childhood psychological abuse were more likely to exhibit behaviors of hostile withdrawal and dominance/intimidation toward their partners. They were also more likely to engage in acts of assault leading to possible injury towards their partner. Participants who experienced physical abuse were more likely to exhibit behaviors of restrictive engulfment, denigration, and hostile withdrawal, and to be more aggressive and engage in acts of assault and be psychologically aggressive towards their partner. Neglect, psychological abuse, and physical abuse were not found to be predictors of participant perpetration in any of the 4 dimensions of emotional abuse in dating relationships.

CONCORDANCE OF ALCOHOL USE AMONG UNDERGRADUATE WOMEN IN DATING RELATIONSHIPS. Michelle K. Crewe & Michelle L. Kelley, Department of Psychology, Old Dominion University, Norfolk, VA 23529-0267. This study examined concordant drinking among 315 undergraduate women between the ages of 18 and 30 and their dating partners. The Substance Abuse Subtle Screening Inventory -3 was used to assess their own and their reports of partner's alcohol use. Four types of couples were expected: alcohol-abusing participants with alcohol-abusing partners (i.e., abuse-concordant couples,  $n = 38$ ), alcohol-abusing participants with a non-alcohol-abusing partner ( $n = 20$ ), non-abusing participants with an alcohol-abusing partner ( $n = 46$ ), and non-alcohol-abusing women and men ( $n = 211$ ). Abuse-concordant couples were compared to other couple types to examine potential differences in relationship satisfaction as measured by the Dyadic Adjustment Scale, quantity of alcohol use as measured by the Daily Drinking Questionnaire, and alcohol consequences as measured by the Rutgers Alcohol Problem Index. As hypothesized, participants who met criteria for alcohol abuse on the SASSI-3 were significantly more likely than expected by chance to report that their dating partner also abused alcohol. Participants in alcohol-concordant couples reported significantly higher alcohol use than participants in other couple types, indicated more alcohol consequences than other couples types except alcohol-abusing women with non-abusing men, and had higher relationship satisfaction than non-abusing women with alcohol-abusing men.

DON'T BE AFRAID TO EXAGGERATE: A STUDY OF EMOTIONAL INTELLIGENCE & PHYSIOLOGICAL MANAGEMENT. Daniel T. Bice, Alexander G. Morris, Victoria Toadvine, Joshua K. Vickstrom, & Hilary E.

Stebbins, Dept. of Psych., Univ. of Mary Washington, Fredericksburg VA 22401. This study investigated the effect of emotional intelligence (EI) and exaggeration of emotional responses on the ability to recover from an aroused state. We hypothesized that participants high in EI would recover faster regardless of condition and that exaggeration would produce a greater discrepancy between the minimum and maximum arousal levels. After completing the Wong-Law Emotional Intelligence Scale, 47 participants (18 men) were randomly assigned to an exaggerate or natural condition and exposed to a 3-minute video clip from *I Know What You Did Last Summer* (1997). Both, physiological arousal and management ability were measured via Galvanic Skin Response. A statistically significant interaction between condition and EI on baseline recovery rate was found such that those high in EI did recover faster than low EI, but only in the non-exaggerate condition. Arguably, exaggeration allows low EI individuals to be more aware of physiological changes and better manage them, but interferes with high EI individuals' natural awareness and management abilities. This finding illustrates that there are potential techniques, such as exaggeration, that can be taught or used to increase emotional intelligence.

BULLYING IN COLLEGE AND ITS EFFECT ON ACADEMIC ACHIEVEMENT. Calesha W. Hayes & Barbara Winstead, Old Dominion University, Norfolk, VA. Bullying and its effects on academic achievement were examined. It was hypothesized that engaging in bullying would negatively correlate with academic achievement in high school and college and that being bullied would negatively correlate with academic achievement in high school and college. Undergraduate Old Dominion University students ( $N=190$ ) were the participants. Participants were presented with a definition of bullying and given an anonymous self-paced questionnaire about academic achievement and bullying others and being bullied in high school and college. The correlation between bullying others in both high school and college with academic achievement was not significant. The correlation between being bullied in high school and college with academic achievement was not significant. A curvilinear relationship was found between the college victim and bully scores and GPA. The medium GPA group was found to have significantly higher bullying and victim scores than the high GPA group, while the low GPA group did not differ significantly from either group. A revised questionnaire or definition of bullying that includes lower levels of bullying and victimization is discussed.

THE INFLUENCE OF SIMULATION STYLE ON PERFORMANCE. Chelsea D. Picot, Rachel R. Phillips, & Poornima Madhavan, Department of Psychology, Old Dominion University, Norfolk VA 23529. Despite the fact that luggage screening involves a dynamic presentation of images, most luggage screening research is conducted with static image presentation. Although some research has suggested that performance with static and dynamic images is equivalent, this has not been addressed in the context of luggage screening. To address this gap, we conducted an experiment to compare luggage screening performance between static and dynamic conditions. Fifty-six students participated for a partial fulfillment of course requirements and were told that they would be playing the role of airline luggage

screeners looking for knives in x-ray images of luggage. The experiment began with a 200 image training session during which participants viewed each image in the center of the screen for 3 seconds. Participants had to indicate if they believed a knife was present before the image timed out. After a short break, participants completed a transfer block of 200 images in one of three conditions: 3 seconds centered, 9 seconds centered, or scrolling. Results revealed that participants had a higher hit rate and a lower false alarm rate in the transfer block than in the training block indicating that their performance improved between training and transfer. In the transfer conditions, participants in the scrolling condition had a significantly lower hit rate than those in the 9 second centered condition. These results indicate that performance with a dynamic versus static image display may differ when it comes to a complex visual search task such as luggage screening and that simulation style may influence results in a meaningful way.

**METHODOLOGICAL CONSIDERATIONS FOR USE OF A MULTIMODAL STROOP-LIKE TASK.** J. Christopher Brill & Elizabeth L. Ferguson, Department of Psychology, Old Dominion University, Norfolk, VA 23529. Development and usage of a multi-modal Stroop-like task for assessing attentional processing requires specific methodological considerations. A task requiring the learning and use of a spatial translation code that supersedes stimulus-response compatibility may facilitate tapping into the same psychological processes involved in performing the classic Stroop task. Information is presented visually, aurally or tactually in a spatial array, and participants respond manually using a spatially congruent response box. Special considerations, however, are required to fairly compare performance for a tri-modal Stroop-like task. Among the considerations are creating highly discernable signals, equating perceptual loudness across sensory modalities, considering response mode, custom fitting the vibrotactile array to each participant, and use of hardware that facilitates accurate measurement of response time. Current research explores the use of this task as a means for assessing differences in processing for multimodal signals. Future research will explore the use of redundantly-coded and spatially incongruent signals.

**SELF-REPORTED AGGRESSIVE DRIVING BEHAVIOR AMONG COLLEGE STUDENTS.** J. Christopher Brill, Lindsey C. Chase, Christina M. Rieger, & Rebecca L. Tiefenback, Department of Psychology, Old Dominion University, Norfolk, VA 23529. The National Highway Transportation Safety Administration identified aggressive driving behavior as a serious hazard to driving safety. We sought to identify potential patterns of aggressive driving behavior among college students using a recently constructed questionnaire: The Aggressive Driving Behavior Questionnaire. The ADBQ is a 20-item self-report questionnaire in which participants rate on a six point Likert-type scale the extent to which they engage in a series of behaviors while driving. Participants completed a basic demographics form and driving history questionnaire, as well as the ADBQ. The results suggest the majority of participants endorse feeling angry at other drivers' behavior, but substantially fewer reported engaging in aggressive acts (e.g., flashing their bright

lights at other drivers, slowing down in front of drivers who are tailgating, and cutting off other drivers). Most participants reported engaging in more passive behaviors, such as shaking their heads at other drivers.

### Statistics

FINDING AN OPTIMAL DESIGN USING PSEUDOFACORS. Mary A. Marion, Department of Statistics, Virginia Polytechnic Institute and State University. This paper was written as a result of an Industrial Systems Engineering project performed at Virginia Tech. This paper reflects an evolving procedure to design an industrial experiment utilizing optimality criteria, AIC statistic and the usual regression/ANOVA model statistics. Discrete factor settings were coded as continuous to utilize response surface methods to find the best settings to reach a specified target. While the industrial example is trivial the characteristics of the project lend themselves to illustrate the complexity of real life applications.

AN INCREMENTAL FORWARD STAGEWISE REGRESSION ALGORITHM FOR DICHOTOMOUS RESPONSE VARIABLES. Adam Sima, Department of Biostatistics, Virginia Commonwealth University. The Incremental Forward Stagewise Regression (IFSR) procedure was developed by Hastie, et al. (2001) as a flexible estimation procedure for fitting penalized linear models. To generalize this procedure, the IFSR estimation method was extended for use with a dichotomous response variable. In particular, a simulation study was used compare both the accuracy in prediction and model fit to similar algorithms that simultaneously fit a model and estimate parameters. The results show that this method is comparable to some commonly used algorithms.

INTRODUCTION TO DISCRETE CHOICE MODELS. Bhaskara S. Ravi and N. Rao Chaganty, Department of Mathematics and Statistics, Old Dominion University. We often encounter with decisions that involve choosing between alternatives or choices such as “which phone to buy” or “which minute plan” to choose or “which brand of shampoo to buy” etc. Interestingly, these decisions not only depend on individual characteristics but heavily on alternatives available. Discrete choice models analyze such choice behavior and these are very popular in economics. This talk aims at introducing very famous McFadden’s conditional logit model and the importance of IIA (Independence of irrelevant attributes) assumption. Also, a review of current trends and challenges in this popular research area are presented.

### Structural Biology, Biochemistry and Biophysics

MECHANISM OF ACTION OF UDP-GALACTOPYRANOSE MUTASE FROM TRYPANOSOMA CRUZI. Michelle Oppenheimer, Ana L. Valenciano, Jun Qi, & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061. *Trypanosoma cruzi* (*T. cruzi*) is the causative agent of Chagas’ disease, which if untreated leads to chronic inflammation of the heart. UDP-galactopyranose

mutase (UGM) is a flavoenzyme that catalyzes the conversion of UDP-galactopyranose (UDP-Galp) to UDP-galactofuranose (UDP-Galf). UGM are important in parasitic pathogenesis and are absent in mammals, making UGM an attractive drug target. The chemical mechanism of UGM is not well understood. We have used steady-state kinetics, rapid-reaction kinetics, and trapping intermediates to better understand the mechanism of function of UGM. A  $k_{\text{cat}}$  value of  $8.1 \pm 0.3$  and a  $K_M$  value of  $43 \pm 7 \mu\text{M}$  were determined for *T. cruzi* UGM (TcUGM) using steady-state kinetics. Spectral change indicative of a predicted flavin iminium ion were detected by rapid reaction kinetics analyses, consistent with a postulated flavin galactose adduct. The intermediate forms at a rate of  $310 \pm 40 \text{ s}^{-1}$ , suggesting this is not the rate limiting step. We have trapped this intermediate and have been able to isolate the adduct by both the HPLC and identify it by mass spectrometry. Lastly, We sought to determine the redox partner for TcUGM. We show that TcUGM can be reduced by NAD(P)H and maintained the reduced state for several catalytic cycles. This activity is unique to eukaryotic UGMs as prokaryotic UGMs do not react with NAD(P)H. Supported by NIH grants GM094469 and AI082542 and the American Heart Association.

PROBING THE MECHANISM OF HIGH-FIDELITY DNA SYNTHESIS USING X-RAY CRYSTALLOGRAPHY. Eugene Wu, Department of Biology, University of Richmond, Richmond, VA. DNA polymerases replicate DNA with higher fidelity than would be expected from free energy differences between complementary and mismatched base pairs. One approach to studying replication fidelity is to determine X-ray crystal structures of reaction intermediates and polymerase complexes with mismatched base pairs. A crystal structure of a guanosine:thymidine triphosphate mismatch in the active site of DNA polymerase I from *Bacillus stearothermophilus* revealed a key intermediate conformation between the "open" and "closed" conformations. The previously unknown "ajar" conformation allows the template to interact with the incoming nucleoside triphosphate and position it relative to the polymerase active site. Complementary dNTPs advance past this conformation to a correctly aligned closed conformation for catalysis, while mismatches are misaligned, leading to substrate release. Other DNA polymerase I family enzymes share features important for the three-state sorting mechanism and are expected to use this mechanism. One homologue with an altered active site sequence may show a preference for the ajar conformation. This homologue's crystal structure has been solved by multiwavelength anomalous dispersion and may lead to confirmation of the three-state or new insights into nucleotide selection by DNA polymerases.

INCORPORATION OF TAUTOMERISM INTO MOLECULAR MODELING; PROGRESS IN DEVELOPING PYRROLE-BASED ANTI-TUBULIN AGENTS. C. Da,<sup>1</sup> G. E. Kellogg,<sup>1</sup> N. Telang<sup>2</sup> & J. Gupton<sup>2</sup>, <sup>1</sup>Virginia Commonwealth University, Richmond VA 23298-0540 <sup>2</sup>University of Richmond, Richmond VA 23173. Tautomerism is a commonly observed chemical phenomenon that involves readily available structural changes of the positions of protons and double bonds. However, alternative tautomeric forms are often ignored in many molecular modeling applications. In docking, inaccuracy in predicting binding affinities can be

related to the failure to consider all possible states including tautomeric states. We are working to incorporate tautomerism into modeling tools based around HINT (Hydropathic INTERactions). A preliminary version of the program has been developed to apply a simple, straightforward algorithm to identify and enumerate tautomeric forms. It is being used in an ongoing project of designing and developing pyrrole-based anti-tubulin agents, to identify and evaluate potential tautomers, and to build quantitative predictive models.

HIGH-THROUGHPUT ASSAY TO IDENTIFY INHIBITORS AGAINST UDP-GALACTOPYRANOSE MUTASE (UGM) FROM *ASPERGILLUS FUMIGATUS*. Jun Qi, Michelle Oppenheimer, & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The flavoenzyme UDP-galactopyranose mutase (UGM) catalyzes the isomerization of UDP-galactopyranose to UDP-galactofuranose, the biosynthetic precursor of galactofuranose (Gal<sub>f</sub>). Gal<sub>f</sub> residues are essential components in the cell wall of pathogens and play vital roles for their virulence. Thus inhibitors of UGM that block the biosynthesis of Gal<sub>f</sub> could lead to novel therapeutics. To date, no eukaryotic UGM inhibitors and only a few prokaryotic UGM inhibitors have been reported. We present the development of a high-throughput fluorescence polarization (FP) assay to identify specific inhibitors of eukaryotic UGM from human pathogenic fungus *Aspergillus fumigatus*. Our FP binding assay demonstrates that specific binding to eukaryotic *Af*UGM were only obtained from UDP-TAMRA chromophores, and a UDP-TAMRA chromophore with  $K_d$  value of  $2.6 \pm 0.2 \mu\text{M}$  was selected as the fluorescent probe in the high-throughput FP assay. The competitive binding assay indicates that this UDP-TAMRA chromophore shares the same binding site with UDP, a known UGM ligand that binds to the active site of UGM. The FP assay was evaluated and displayed excellent  $Z'$  factor ( $0.79 \pm 0.01$ ) and good tolerance to DMSO. Nine compounds were screened in this system, and one compound was identified as *Af*UGM ligand. This compound was further confirmed to inhibit the activity of *Af*UGM in *Af*UGM activity assay, indicating that the compounds identified by our FP high-throughput screening system are inhibitors of *Af*UGM. Supported by a NIH grant RO1-AI082542 (R. Tarleton PI).

HOMOLOGY MODELS OF C-C CHEMOKINE TYPE-5 RECEPTORS. ARE BOUND WATERS IMPORTANT IN BINDING SITES OF MEMBRANE BOUND RECEPTORS? Saheem A. Zaidi, Philip D. Mosier, Yan Zhang & Glen E. Kellogg, Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298. HIV-1 uses two plasma membrane receptors, CD4 and a co-receptor, to facilitate its entry into the target plasma membrane. Depending upon the type of virus, this co-receptor can either be C-C chemokine receptor type-5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4). Studies suggest that CCR5 plays a dominant role in early stages of infection. Many allosteric antagonists of CCR5 are known to inhibit fusion/entry process and subsequent infection of HIV-1. In the present study we explored an allosteric binding site of CCR5 and the possible role protein-bound water molecules may play in the interaction. A homology model of CCR5 was built using recently crystallized CXCR4 as a template, followed by virtual docking of maraviroc, a known allosteric antagonist. Mutagenesis studies



from the literature agreed with the docking pose obtained. Possible protein-bound water sites were generated and the antagonist was re-docked. According to our model there are at least two important protein-bound waters. One of the bound waters forms a hydrogen bond bridge between the ligand and the protein while the second bound water is displaced due to ionic-bridge formation between the ligand and the protein, and thus increasing the binding affinity due entropic gain resulting from disruption of water network in the unliganded receptor.

**ANALYSIS OF POST-TRANSLATIONAL MODIFICATIONS OF SIKE FOLLOWING DOUBLE-STRANDED RNA STIMULATION.** Charlotte F. Roberts, James D. Marion, R. Jason Call and Jessica K. Bell, Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. The innate immune system is the body's first line of defense against infectious agents. Essential to this response are cellular mechanisms that recognize, sequester and eradicate these invading organisms. Toll-like receptor 3 (TLR3), a pathogen recognition receptor, is stimulated by the viral genomic material double stranded RNA (dsRNA). TLR3 stimulation initiates a signaling cascade that leads to the production of type I interferon. Critical to this signaling pathway is a kinase complex, NAPI (NAK associated protein 1)-TBK1 (TANK Binding Kinase 1)-IKK $\epsilon$  (I $\kappa$ B kinase epsilon), which leads to the phosphorylation of IRF3 (interferon regulatory factor) and IRF7 and production of IFN $\beta$ . To control this activity, SIKE (Suppressor of IKK $\epsilon$ ) acts as a physiological inhibitor of IKK $\epsilon$  and TBK1 activity through an undefined mechanism. The role of post-translation modifications to control SIKE function was examined. FLAG-tagged SIKE DNA was transiently transfected into HEK293 followed by dsRNA stimulation for 24 h. Cell lysates were harvested, SIKE immunoprecipitated using anti-FLAG antibody agarose beads. Immunoblot analysis showed serine phosphorylation state following dsRNA stimulation. Using in vitro kinetic assays, TBK1 mediated SIKE phosphorylation. These results suggest that upon pathway stimulation and TBK1 activation, TBK1 phosphorylates SIKE signaling SIKE's release from the kinase complex. Funding provided in part by the American Cancer Society.

**SUPPRESSOR OF IKK-EPSILON IS A MIXED TYPE INHIBITOR OF THE TYPE I INTERFERON RESPONSE.** James D. Marion, Charlotte F. Roberts, R. Jason Call, and Jessica K. Bell, Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. Kinases act in signaling pathways to propagate the cellular response by phosphorylation of specific targets that trigger downstream events that alter the cell's transcriptional program. To control kinase activity, the cell has developed an intricate series of protein interactions that are required for activation or mediate inhibition. In innate immunity, viral-derived ligands activate innate immune receptors to initiate an anti-response including production of type I interferons. Downstream of these receptors, a critical kinase complex, NAPI (NAK associated protein 1)-TBK1 (TANK Binding Kinase 1)-IKK $\epsilon$  (I $\kappa$ B kinase epsilon) phosphorylates of the transcription factor, IRF3 (interferon regulatory factor) and IRF7, which leads to the production of IFN-beta. To control this activity, SIKE (Suppressor of IKK-epsilon) acts as a physiological inhibitor the kinase activity through an undefined mechanism. Our goal is to define

the inhibitory mechanism of SIKE in the kinase reaction. Using an in vitro kinase assay, the Michaelis constant for IRF3 phosphorylation was determined (5 micromolar). Upon addition of increasing concentrations of SIKE, a decrease in  $V_{max}$  for IRF3 phosphorylation was observed with minimal change in  $K_m$ . Further analysis showed that SIKE functioned as a mixed type inhibitor with an approximate  $IC_{50}$  of 450 nM. The effect of SIKE phosphorylation on inhibitory function is currently under study. Funding provided in part by the American Cancer Society.

STRUCTURE BASED PREDICTIVE MODELS FOR PROTEIN POST-TRANSLATIONAL MODIFICATIONS. M. Zhang<sup>1</sup>, V. A. Yakovlev<sup>2</sup>, R. B. Mikkelsen<sup>2</sup> & G. E. Kellogg<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, VA 23298 <sup>2</sup>Department of Radiation Oncology, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298. Nitric oxide (NO) has long been identified as a diffusible signaling molecule, but only until recent years had gained prominence as an inducer of redox-based protein post-translational modifications (PTMs). NO-dependent PTM is achieved, in a large part, through cysteine S-nitrosylation, the covalent addition of the NO moiety to a reactive cysteine thiol (other modes of action also exist, such as tyrosine nitration). Over the last decade, the number of reported substrates for S-nitrosylation has grown exponentially, and are shown to participate in a wide range of biological processes, however, the precise mechanism of S-nitrosylation is still elusive, the only certainty is that targeting of S-nitrosylation between proteins, and especially between Cys residues within substrate proteins reflects the interactions of a number of determinants of specificity. In an attempt to untangle these determinants, we have developed computational methods to build models of protein post-translational modifications based on 3D structural features, which have been successfully applied to predict tyrosine nitration. In the current study, we are developing models that we hope can accurately predict Cys S-nitrosylation. Preliminary analysis with 821 cysteines has proven to be promising, some interesting interactions between structural features have been identified with a logistic regression model, and a much simpler but more accurate model was built with a novel ant-colony algorithm.

**VIRGINIA JUNIOR ACADEMY OF SCIENCE  
MAY 25, 2011, 70TH ANNUAL MEETING  
UNIVERSITY OF RICHMOND  
RICHMOND, VIRGINIA**

**Agriculture and Animal Science**

Honorable Mention:	Kintora Chapman Central Virginia Governor's School
Honorable Mention:	Haven Headley Chesapeake Bay Governor's School
Honorable Mention:	Mallory Banton Hanover High School
Third Place:	Ryan Boles Chesapeake Bay Governor's School
Second Place:	Clayton Geipel and Jason McAleese SMT at Godwin High School
First Place:	Jennifer Heyward Shenandoah Valley Governor's School

**Animal Behavior**

Honorable Mention:	Thomas Coleman, Kevin Gilbert and Rahim Islam Chesapeake Bay Governor's School
Honorable Mention:	Stuart Geipel Deep Run High School
Honorable Mention:	Sarah Graham and T.A. Njuyen Thomas Jefferson High School for Science and Technology
Third Place:	Ashley Wayne Central Virginia Governor's School
Second Place:	Natalie Hahn SMT at Godwin High School
First Place:	Hannah Heimer Central Virginia Governor's School

**Botany**

Honorable Mention:	Adam Grim George H. Moody Middle School
Honorable Mention:	William O'Brochta Roanoke Valley Governor's School
Honorable Mention:	Jagannath Kadiyala Godwin High School
Third Place:	Tyler Laredo Washington-Lee High School
Second Place:	Brooke Baker and Amber Brown Chesapeake Bay Governor's School
First Place:	Erik Zorn Roanoke Valley Governor's School

**Chemistry A**

Honorable Mention:	Corinne Moorefield Ocean Lakes High School
Honorable Mention:	Kaixin Chen Godwin High School
Honorable Mention:	Jee In Seo Thomas Jefferson High School for Science and Technology
Third Place:	Joel Goodloe Hanover High School
Second Place:	Arjun Jaini Lee-Davis High School
First Place:	Prasana Joshi SMT at Godwin High School

**Chemistry B**

Honorable Mention:	Victoria Nguyen Bishop O'Connell High School
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Honorable Mention:	Sathya Areti and Garrick Sin Henrico High School
Honorable Mention:	Kevin Zhan George H. Moody Middle School
Third Place:	Ashley Canaan George H. Moody Middle School
Second Place:	Priya Sarkar Henrico High School
First Place:	Soyun Kim Central Virginia Governor's School

**Computer Science**

Honorable Mention:	Kelvin Chen Deep Run High School
Honorable Mention:	Kyla Bouldin Washington-Lee High School
Honorable Mention:	Zuahear Mansiv Washington-Lee High School
Third Place:	Bradley Lyman Mountain Vista Governor's School
Second Place:	Christian Dolliff Washington-Lee High School
First Place:	Luke Faraone Yorktown High School

**Consumer Science A**

Honorable Mention:	Jessica Hawkins Shenandoah Valley Governor's School
Honorable Mention:	Jessica Evans Shenandoah Valley Governor's School
Honorable Mention:	Eileen Breslin Washington-Lee High School

Third Place:	William Joyce IV Mountain Vista Governor's School
Second Place:	Tanner Westmoreland Mountain Vista Governor's School
First Place:	Amna Adrees Shenandoah Valley Governor's School

**Consumer Science B**

Honorable Mention:	Michelle Heath Central Virginia Governor's School
Honorable Mention:	Mark Feinberg Thomas Jefferson Middle School
Honorable Mention:	Frank Gaskins and Mischa Turner Chesapeake Bay Governor's School
Third Place:	Ciara King and Mariah Martin Chesapeake Bay Governor's School
Second Place:	Kiersten Garcia Central Virginia Governor's School

**Earth and Space Science**

Honorable Mention:	Purnima Ghosh George H. Moody Middle School
Honorable Mention:	Emily Hastings Hanover High School
Honorable Mention:	Alan Booth Hampton High School
Third Place:	Ramsey Natour Central Virginia Governor's School
Second Place:	Benjamin Wong George H. Moody Middle School
First Place:	Zoey Dorman Central Virginia Governor's School

**Engineering**

Honorable Mention:	Paul DiMartino George H. Moody Middle School
Honorable Mention:	Samantha Marquez Maggie L. Walker Governor's School
Honorable Mention:	Nick Ladocsi Mathematics and Science High School at Clover Hill
Third Place:	Liz Carpenter Central Virginia Governor's School
Second Place:	Akshay Deverakonda Thomas Jefferson High School for Science and Technology
First Place:	Andrew Gatow, Sherwood Lin and Robert Shock Blacksburg High School

**Environmental Science A**

Honorable Mention:	Ariel Bobbet Washington-Lee High School
Honorable Mention:	Sarah Goodman Washington-Lee High School
Honorable Mention:	Angela Mestre and Emilia Zevallos Washington-Lee High School
Third Place:	George Maurakis Mathematics and Science High School at Clover Hill
Second Place:	Casey Cooke Mountain Vista Governor's School
First Place:	Darwin Li Thomas Jefferson High School for Science and Technology

**Environmental Science B**

Honorable Mention:	Akshita Sharma George H. Moody Middle School
Honorable Mention:	Jordan Word Robious Middle School

Honorable Mention:	Hannah Patrick Mountain Vista Governor's School
Third Place:	Aolin Zhang JR Tucker High School
Second Place:	Mark Pfeifer Mountain Vista Governor's School
First Place:	John-Reid Ryan Hanover High School

**Environmental Science C**

Honorable Mention:	Margaret Anne Self Chesapeake Bay Governor's School
Honorable Mention:	Samantha Schwartzkopf George H. Moody Middle School
Honorable Mention:	Sarah Sisson Chesapeake Bay Governor's School
Third Place:	Connor Wood George H. Moody Middle School
Second Place:	Emma Hollowell Chesapeake Bay Governor's School
First Place:	Kendall Trivett Chesapeake Bay Governor's School

**Environmental Science D**

Honorable Mention:	Seth Diggs Chesapeake Bay Governor's School
Honorable Mention:	Hannah Wallace Chesapeake Bay Governor's School
Third Place:	Rebecca Walker Chesapeake Bay Governor's School
Third Place:	Andrea Green and Catherine Mitchell HB Woodlawn High School



Second Place: Valentina Lohr  
Williamsburg Middle School

First Place: Rachel Corrigan  
Chesapeake Bay Governor's School

**Genetics and Cellular Biology**

Honorable Mention: Reshini Premaratne  
George H. Moody Middle School

Honorable Mention: Rhiannon Edwards  
Gildersleeve Middle School

Third Place: Anant Kharkar  
George H. Moody Middle School

Second Place: Radha Venkatesan  
York High School

First Place: Nadine Nikolova  
Deep Run High School

**Mathematics**

Second Place: Yeji Kim  
Deep Run High School

First Place: Agustey Mongia  
George H. Moody Middle School

**Medicine and Health A**

Honorable Mention: Diana Otaya  
Mountain Vista Governor's School

Honorable Mention: Paul Girgis  
Godwin High School

Honorable Mention: Sayantane Das  
Godwin High School

Third Place: Yanni Hedjar  
Shenandoah Valley Governor's School

First Place: Harsh Patolia  
Roanoke Valley Governor's School

**Medicine and Health B**

Honorable Mention:	Aejoo Hong Central Virginia Governor's School
Honorable Mention:	Michael Bonini Bishop O'Connell High School
Honorable Mention:	Elena Anderson Southwest Virginia Governor's School
Third Place:	Elizabeth Hilbert Appomattox Regional Governor's School
Second Place:	Jason Cui Langley High School
First Place:	Riley Ennis Thomas Jefferson High School for Science and Technology

**Medicine and Health C**

Honorable Mention:	Avery Mahaney Southwest Virginia Governor's School
Honorable Mention:	Elizabeth Levit George H. Moody Middle School
Honorable Mention:	Sehaj Khara JR Tucker High School
Third Place:	Nathan Lin George H. Moody Middle School
Second Place:	Sreemoyee Som Roanoke Valley Governor's School
First Place:	Sameer Sarkar Maggie L. Walker Governor's School

**Microbiology A**

Honorable Mention:	Angela Huckstep Hanover High School
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## VJAS AWARDS

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Honorable Mention:	Ao Zhang SMT at Godwin High School
Honorable Mention:	Sarah Jensen Yorktown High School
Third Place:	Ashley Gartin Shenandoah Valley Governor's School
Second Place:	Jacob Melton Math and Science High School at Clover Hill
First Place:	Levi Helm Roanoke Valley Governor's School

### Microbiology B

Honorable Mention:	Sruthi Jayaraman Robert Frost Middle School
Honorable Mention:	David Lanman Thomas Jefferson Middle School
Honorable Mention:	Emily Lodge Bishop O'Connell High School
Third Place:	Monika Payerhin Central Virginia Governor's School
Second Place:	Leigh Anna Morris and Marian Walker Chesapeake Bay Governor's School
First Place:	Coly Elhai George H. Moody Middle School

### Physical Science A

Honorable Mention:	Kayla Sell George H. Moody Middle School
Honorable Mention:	Rebecca Merriman-Goldring Swanson Middle School
Honorable Mention:	Jacqueline Anders Swanson Middle School

Third Place: Ryan Lucia  
Stonewall Jackson Middle School

Second Place: Claire Spaulding  
Swanson Middle School

First Place: Seth Peacock  
Stonewall Jackson Middle School

#### **Physical Science B**

Honorable Mention: Jake Cui  
Kilmer Middle School

Honorable Mention: Stephen Russell  
George H. Moody Middle School

Honorable Mention: William Deng  
George H. Moody Middle School

Third Place: Myra Ahmad  
George H. Moody Middle School

Second Place: Mihir Paithane  
George H. Moody Middle School

First Place: Caroline Fudala  
James River High School

#### **Physics A**

Honorable Mention: Molly Huss  
Shenandoah Valley Governor's School

Honorable Mention: Juliana Bain  
Swanson Middle School

Third Place: Thomas Varley  
Yorktown High School

Second Place: Sarah Thibodeau  
Shenandoah Valley Governor's School

First Place: Ellen Korcovelos  
SMT at Godwin High School

**Physics B**

Honorable Mention:	Austin Holmes Central Virginia Governor's School
Honorable Mention:	Melissa Havel Ocean Lakes High School
Honorable Mention:	David Carson Roanoke Valley Governor's School
Third Place:	Conner Peters Central Virginia Governor's School
Second Place:	Thomas Sheehy Thomas Jefferson Middle School
First Place:	Swapnil Pande George H. Moody Middle School

**Psychology-General**

Honorable Mention:	Maya Ravindran Douglas S. Freeman High School
Honorable Mention:	Supreeti Sharma Mountain Vista Governor's School
Third Place:	Tori Auerhan HB Woodlawn High School
Second Place:	Megan Geboski Mountain Vista Governor's School
First Place:	Lakshmi Bodapati JR Tucker High School

**Psychology-Learning and Perception A**

Honorable Mention:	Evelyn Strobe Roanoke Valley Governor's School
Honorable Mention:	Megan Wentworth Roanoke Valley Governor's School
Honorable Mention:	Ann Marie May Gildersleeve Middle School

Third Place:	Amanda Hinckle and Alicia Talley Deep Run High School
Second Place:	Elizabeth McDonald Yorktown High School
First Place:	Michael Nguyen and Kevin Zhou Thomas Jefferson High School for Science and Technology

**Psychology-Learning and Perception B**

Honorable Mention:	Diamond Pollard Central Virginia Governor's School
Honorable Mention:	Bria Atkins Central Virginia Governor's School
Honorable Mention:	Preston Rogers Central Virginia Governor's School
Third Place:	Eugene Jeong and Peter Pak Deep Run High School
Second Place:	Drew Precious Central Virginia Governor's School
First Place:	Sarah Murphy Douglas S. Freeman High School

**Psychology-Social**

Honorable Mention:	Katherine Mireles Ocean Lakes High School
Honorable Mention:	Kelly Johnson Shenandoah Valley Governor's School
Honorable Mention:	Lara Phillips Yorktown High School
Third Place:	Leah Dillard George H. Moody Middle School
Second Place:	Alison Eddins George H. Moody Middle School

First Place: Margaret Doyle  
Williamsburg Middle School

**Statistics**

Honorable Mention: Kerri Anderson  
Goochland High School

Honorable Mention: Brittany Moncure  
Deep Run High School

Third Place: Alison Hauslee  
SMT at Godwin High School

Second Place: Eddie Hanks  
Pulaski High School

First Place: Nitin Iyer  
Ocean Lakes High School

**Zoology**

Honorable Mention: Climothy Morris  
Chesapeake Bay Governor's School

Honorable Mention: Alexander Schmitt  
Mountain Vista Governor's School

Honorable Mention: Abhya Panya Vij and Jennifer Wang  
Thomas Jefferson High School for Science and Technology

Third Place: Andrew Leffler  
George H. Moody Middle School

Second Place: Andre Desruelle  
HB Woodlawn High School

First Place: Natasha Sheybani  
Appomattox Regional Governor's School

### Special Awards

#### Botany Section Award

Botany Section Award, given by the Botany Section of the VAS, to the best paper on a botanical subject.

Erik Zorn  
Roanoke Valley Governor's School

#### Mathematics Award

Mathematics Award for the paper that evidences the most significant contribution in the field of Mathematics.

Agustey Mongia  
George H. Moody Middle School

#### Statistics Award

Statistics Award for the paper that evidences the most significant contribution in the field of Statistics.

Nitin Iyer  
Ocean Lakes High School

#### Speleological Society Award

Given by the Richmond Area Speleological Society for outstanding research addressing karst or topics related to speleology (bats, caves, carbonate geology, paleoenvironments, limestone fossils, sinkholes, etc.

Rachel Marzen  
Thomas Jefferson High School for Science and Technology

#### Smith Shadomy Infectious Disease Award

Given to commemorate Dr. Smith Shadomy by the Virginia Chapter of the National Foundation for Infectious Diseases to the paper that evidences outstanding research in the field of infectious diseases.

Prasana Joshi  
SMT at Godwin High School

#### Roscoe Hughes Award

Roscoe Hughes Award for the best paper in the field of Genetics.

Rhiannon Edwards  
Gildersleeve Middle School

#### Rodney C. Berry Chemistry Award

Rodney C. Berry Chemistry Award for the paper that evidences the most significant contribution in the field of chemistry.

Prasana Joshi  
SMT at Godwin High School



**Dr. and Mrs. Preston H. Leake Award in Applied Chemistry**

The Dr. and Mrs. Preston H. Leake Award in Applied Chemistry will be given to the author of a research paper which best exemplifies how chemicals, chemical principles, or chemistry have been used, are used, or might be used to enhance or even to save life.

Riley Ennis

Thomas Jefferson High School for Science and Technology

**Catesby Jones - Russell J. Rowlett Award**

Catesby Jones - Russell J. Rowlett Award for the Best Research Paper of the Year.

Michael Nguyen and Kevin Zhou

Thomas Jefferson High School for Science and Technology

**Virginia Sea Grant College Program**

Virginia Sea Grant College Program Award is given by the Virginia Sea Grant College Program for outstanding marine or coastal research.

Rachel Corrigan

Chesapeake Bay Governor's School

**American Cancer Society Award**

American Cancer Society Award - This award is to recognize outstanding science papers related to cancer research. These awards are provided by the American Cancer Society.

Riley Ennis

Thomas Jefferson High School for Science and Technology

Sayantane Das

Godwin High School

Radha Venkatesan

York High School

**Gamma Sigma Delta Award (Agriculture)**

The Gamma Sigma Delta Award (Agriculture). Presented by the VPI & SU Chapter of the Honor Society of Agriculture. This award is presented in recognition of excellence in research dealing with application of new technologies and/or concepts in agriculture forestry, or veterinary medicine.

Jennifer Heyward

Shenandoah Valley Governor's School

**Dominion - W.W. Berry Award**

Dominion - W.W. Berry Award. This award is given by Dominion Virginia Power in honor of Mr. W. W. Berry who was a past Chairman of the Board of VA Power.

Andrew Gatow, Sherwood Lin and Robert Shock

Blacksburg High School

**Joyce K. Peterson Award**

The Joyce K. Peterson Award is presented for the outstanding paper by a middle school

student. It is presented in honor of Mrs. Joyce K. Peterson who has been an outstanding teacher in the Arlington County Schools.

Valentina Lohr  
Williamsburg Middle School

**Ann M. Hancock Award**

The Ann M. Hancock Award - This award is given to the best paper in cellular biology and is given in memory of Anne Hancock who retired from Patrick Henry High School in Hanover County and who gave many years of service to the Jr. Academy not only by teaching but also serving on the Jr. Academy Committee.

Nadine Nikolova  
Deep Run High School

**Dorothy Knowlton Award**

Dorothy Knowlton Award - This award is given to the best paper in the Consumer Science section(s) and is given in honor of Dorothy Knowlton, former Science Coordinator of Arlington County Schools.

Lukas Mohnach  
Chesapeake Bay Governor's School

**VABE Award**

outstanding research in Zoology VABE Award - This award is presented by the Virginia Association of Biology Educators and is given for outstanding research in the Zoology section.

Natasha Sheybani  
Appomattox Regional Governor's School

**Virginia Museum of Natural History Award**

Virginia Museum of Natural History Award - Presented by the Friends of the Virginia Museum of Natural History in recognition of significant contribution in the study and interpretation of Virginia's Natural Heritage.

Rachel Corrigan  
Chesapeake Bay Governor's School

**AAAS Award and Honorary Membership**

The AAAS Award is given for presenting an outstanding paper at this symposium. This student will also receive an honorary membership to AAAS and a cash award of \$200.

Prasana Joshi  
SMT at Godwin High School

**VAS Honorary Membership**

One year's membership, without cost, to the Virginia Academy of Science including a subscription to the Virginia Journal of Science will be awarded.

Ana O'Harrow  
Yorktown High School

**Bethel High School Scholarship**

Bethel High School Scholarship - This \$1,000 Scholarship Award comes from the interest earned from a \$10,000 endowment contributed by the students of Bethel High School, Hampton, Va., over a two year period. This award is based on both the student's presentation and paper.

Rachel Corrigan  
Chesapeake Bay Governor's School

**Henry MacKenzie Environmental Scholarship (VEE)**

Henry MacKenzie Environmental Scholarship - This \$5,000 scholarship will be awarded to the student whose paper evidences the most significant contribution in the field of Environmental Science dealing with the James River Basin and Chesapeake Bay. The Virginia Endowment and VJAS offer this scholarship in tribute to the outstanding and generous services of Judge Henry W. MacKenzie, Jr., one of the founding directors who has a great interest in the James River and the Chesapeake Bay.

Alan Booth  
Hampton High School

**Frances and Sydney Lewis Environmental Scholarship (VEE)**

Frances and Sydney Lewis Environmental Scholarship: A \$14,000 scholarship (\$3,500 per year for four years) for the best effort by a student in grades 9 to 12 in the field of environmental science. This scholarship is in the name of Frances and Sydney Lewis and is given by the Virginia Environmental Endowment.

Sarah Sisson  
Chesapeake Bay Governor's School

**E.C.L. Miller Science Teacher of the Year Award**

E.C.L. Miller Science Teacher of the Year Award is given to an outstanding science teacher.

Tim Criner  
Gildersleeve Middle School

**Distinguished Service Award**

VJAS Distinguished Service Award, most prestigious award given by the VJAS, is presented to a person for exceptionally outstanding service to the VJAS.

Michael Lovrencic  
Yorktown High School

## NOTES